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CYTOLOGICAL STUDIES OF PLASMODIUM: THE MALE GAMETE

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Although extensive investigations have been carried on concerning the malaria parasite, *Plasmodium*, little information is available as to the fundamental cytological problems of this form. For this reason, work was begun in the summer of 1943 in an effort to follow cytologically the various stages of the life cycle. In spite of the smallness of the chromosomes and some peculiarities in nuclear behavior, some stages are easily seen and quite definite as the photographs show.

This paper is limited to consideration of the development of the male gametes from the mature gametocytes; very interesting and suggestive results having been obtained in this phase of the study.

One difficulty in determining the chromosome number of *Plasmodium* is the fact that in this form, as in some other *Protozoa*, e.g. *Chilodonella* (MacDougall, 1925, and 1936) the chromosomes go into and come out of the resting stages in both mitosis and meiosis paired and so fused as to make the chromosome number appear to be half of what it really is. Bearing in mind the size of the malaria parasite, it is not strange that long study and patience are required for this work.

How the gametocytes originate cannot be explained at this time. Several authors have shown that both micro- and macrogametocytes are distinguishable from other types of *Plasmodium* cells in the very early stages, being found in the internal organs as well as in the peripheral blood. Boyd (1935), in a very interesting study of *P. vivax* cells present in his material, arranges the parasites in 5 series, designated as

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A, B, C, D, and E and suggests that the cells in series A. may be regarded as the stem cells for the other series. Probably his findings and suggestions are correct but the manner in which the pregametes that he mentions are differentiated remains to be worked out.

Another complication is the *exoerythrocytic cycle.* The nuclear situation of this cycle will have to be worked out before the cytological picture of the life cycle of *Plasmodium* is complete.

MATERIALS AND METHODS

The microscope used in this work was a B & L binocular research type with 12.5 paired compensating oculars and a 2 mm apochromatic oil immersion lens. The photographs were taken with the same microscope, using a single tube and a 15× ocular with the camera set 10 inches from the eyepiece. The initial magnification is, therefore, 1350. The smaller pictures were enlarged approximately 3 times and the larger ones approximately 5 making the magnification legends as indicated in the figures.

Pen and ink sketches made by use of transfer paper are included to make clear the critical points in the unretouched photographs. This allows the reader to judge the situation first hand. Elaborate plates often represent only the author's interpretation of the material.

Over a thousand slides were made by various methods, the material being obtained mainly in the State Hospital in Columbia, S. C., from patients infected with malaria therapeutically. Some good material was obtained, however, from the Moore General Hospital, Asheville, N. C.

Dry smears were made of all stages for a check but all work was done from preparations fixed wet. The blood smears were put in damp chambers, then killed at intervals of 5–10–15–20–25–30 minutes, the 15 and 20-minute slides being the most generally useful, though, in some cases, the 5-minute slides showed exflagellation (formation of male gametes). The mosquito material was obtained by allowing mosquitoes reared in the insectary to feed on malaria patients showing a fair or good gametocyte count, these being killed at intervals and the stomach dissected out and fixed. Many standard fixatives and stains were used. For use with the Giemsa stain, the fixative most generally useful was Wenrich's modification of Schaudinn's fluid (McClung, page 533); for use with the hematoxylin stain, Carnoy's or Gilson's fixatives appeared to give better results. It was found that in the *falciparum* studies, it was necessary to depigmentize the parasites. The schedule followed for the hematoxylin studies was that of Sinton and Mulligan (1930). For the Giemsa stain the long method recommended by McClung (1937 page 536) was the most satisfactory. The dry slides were handled according to the technique recommended by Miss Wilcox (1941).

The Microgametocyte

As has been described by other workers many times, the chromatin in the nuclei of the mature male gametocytes of both *falciparum* and *vivax* is granular and diffuse, a fact that allows for the ready identification of these cells. Even after the granules

PLATE I

Fig. 1, A and B. Granules arranged in rows in the male gametocyte. This probably represents synapsis of 4 pairs of leptotene threads (*falciparum*). × 4050+.

Fig. 2, A and B. Pairing of 4 pairs of chromosomes arranged end to end. Note single body at the anterior end; note also space between the first and second pairs of chromosomes (*falciparum*). × 4050+.

Fig. 3, A and B. "Thick thread" or pachytene stage. × 6750+.

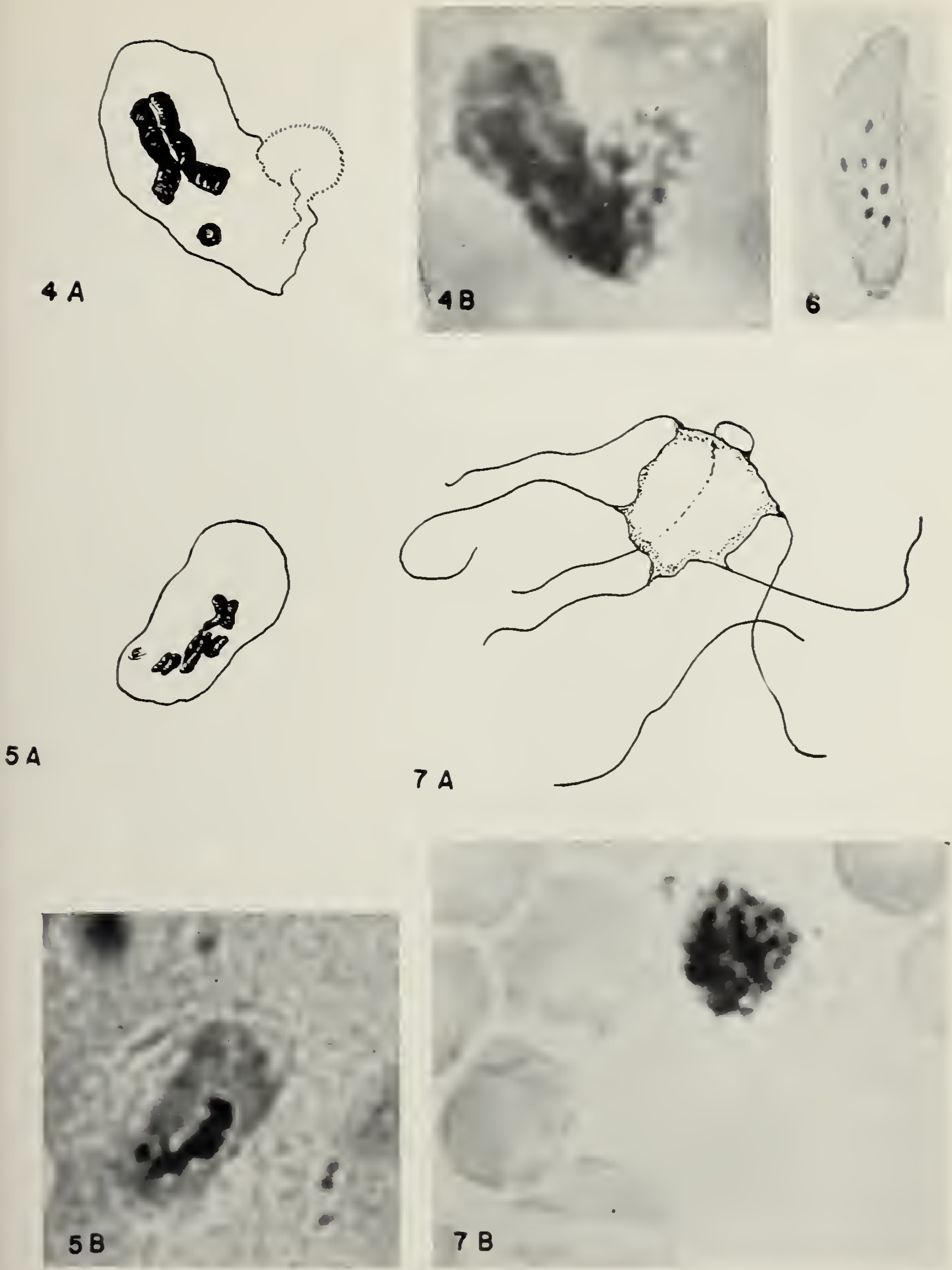


Plate II

Fig. 4, A and B. Separation of pachytene threads. Nucleolus-like body is present (*falciparum*). $\times$ 6750+.

Fig. 5, A and B. Separation of 4 pairs of chromosomes from the pachytene stage. Note heterotypic pair at anterior end (falciparum). $\times$ 4050+.

Fig. 6. Thompson's figure of 8 split chromosomes (falciparum). $\times$ 4050+.

Fig. 7, A and B. Formation of the cytoplasmic element of the male gamete. Note that 1 gamete has broken off without receiving the chromatin element. It is, therefore, *sterile* (*vivax*). $\times$ 4050+.

have lined up, the background of the nucleus stains red with the Giemsa stain. This reaction is due to the fact that the nucleus "purifies" itself by the extrusion of material that stains a lighter red than does the chromatin when destaining is done properly. With hematoxylin, it stains almost the same way as the chromatin but in properly differentiated slides, the stain is somewhat lighter than that of chromatin. With the Giemsa stain, the cytoplasm of the microgametocyte stains a light blue in contrast to the deeper blue stain of the macrogametocyte.

Development of the Microgamente

In the early stages of development, the granules in the nuclei of the microgametocytes arrange themselves in longitudinal rows. For a time the picture is confused on account of the material that has been extruded from the nucleus. In the end there are 4 rows; this is definite, as Figure 1 shows, but it is certain that there is a larger number earlier and that these earlier granular threads are much thinner. They are very probably the leptotene threads and Figure 1 may possibly be a zygotene stage, that is, a synapsis of the leptotene threads. Some preparations of my own suggest the larger number of threads and some of Cowdry's figures show this also (Anderson and Cowdry, 1928). The method of the formation of the male gamete described by them, is very different, except in the first stages, from that shown by my material. However, Anderson and Cowdry worked with different species (*P. kochi*). If Figure 1 represents the zygotene stage, then there are several stages missing between Figure 1 and Figure 2.

At first glance, it would seem that Figure 2 represents a pairing of 2 strands but a study of later stages shows that this is not so. Reference to Figure 5 suggests that Figure 2 represents 4 chromosomes paired not only side by side (parasynapsis) but arranged end to end. Note that the first pair is separated somewhat from the other 3 pairs; this is a heterotypic pair that is easily recognizable later when the chromatin elements are ready to enter the cytoplasmic element of the immature sperm. It is also evident when the chromosomes come out of synapsis, Figure 5. Note also the formed body that moves out from behind the first paired mass. Although it divides later, its role is unknown as yet. Its mode of origin (from possible satellites) suggests that it is a nucleolus-like body but its behavior is obscure.

In Figure 3 is shown a "thick thread" or pachytene stage. Presumably this follows the stage shown in Figure 2. It will be noted that in addition to the longitudinal line indicating parasynapsis, there are indications as to where transverse separation will take place also. When the thick thread stage breaks up, 4 pairs of chromosomes are seen and often each chromosome appears split also. In my material, however, I have never found the 8 paired (or split?) chromosomes shown by Thomson (1932) Figure 6. This stage probably exists though I have not found it as yet. It is very probable that there are several stages that I have not been able to locate that would explain the composition of the paired chromatin mass that enters each male gamete. As it stands, however, these stages have not been found after long search. Very probably they develop very rapidly and exist a very short time. Further work is planned along this line.

Cytoplasmic Element of the Male Gamete

The description by Raffaele (1939) of the formation of the flagella is essentially correct. There is an outpushing of the cytoplasm to form the motile part of the male gamete, Figure 7. The double nature of this structure is shown by the abnormal flagellum in Figure 9. Sometimes when the chromatin element enters the cytoplasmic element, it may push "blobs" of cytoplasm in front of it or pull them along behind. This figure is included to show the double nature of the flagellum for most of its length; it is single about one-fourth of the way from the tip.

Raffaele (1939) and other workers have pointed out that the male gametocyte very often slips out of its capsule, giving the appearance of 2 parasites being present. This does not always happen and does not seem necessary to development. Probably it is not a normal condition but may be occasioned by change in pressure when slides are even slightly dried. Figures 9 and 10 show male gametocytes partly out of their capsules.

The Chromatin Element

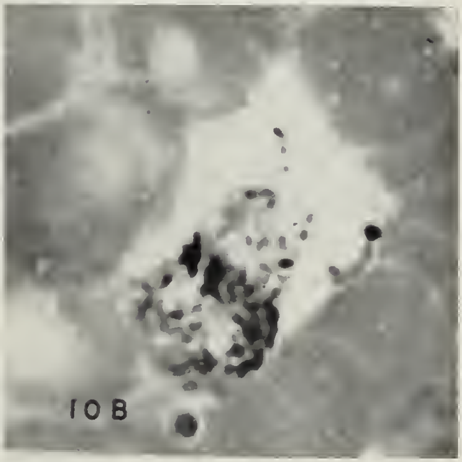
By the time the flagella have been formed, the chromatin masses are ready to enter. It is certain that only one paired mass enters each gamete, but this may easily represent 4 chromosomes. In *Chilodonella* where stages were more easily followed, 4 chromosomes appeared as 1 pair when going into the resting stage in both mitosis and meiosis stages. If this be true, a third division, common in ciliates during meiosis, must occur. It has not been found. Figure 10 shows 4 pairs of chromatin masses, a heterotypic paired mass being shown on the left. Since this heterotypic pair is seen in several stages during development, one naturally thinks that it may have something to do with sex determination. If there is a separation of the chromosomes as shown by Thomson (1932), then the paired masses in Figure 10 would represent a rearrangement of the chromosomes in preparation for entrance into the sperm; they would not have the same "line up" as in Figure 5 which shows the chromosomes emerging from the pachytene stage. Until the reduction division is cleared up, however, just what the paired masses in these 2 figures represent is open to question. Since 4–8 flagella are usually formed, it is more than probable that the clear picture of 4 paired masses is not the whole story. In Figure 11, two masses have entered the cytoplasmic elements, two paired masses are clearly seen in the center of the gametocyte and in the posterior part of the cell is some chromatin which may be another paired mass more fused than the other two masses. This may or may not be extruded chromatin; it does not appear so. Also, some sperm may have already left the gametocyte. Although hundreds of exflagellating gametocytes have been examined the number of chromatin paired masses that are formed has not been determined due to the fact that when the paired chromatin masses are ready to enter the cytoplasmic elements, some enter early and break off and others are delayed in moving into the sperm. For this reason one can never be sure just how many there are. The number most often seen is 4 as shown in Figure 10.

When the formed male gametes break off, the head appears solid, as shown in

8

9

10 A



10 B

PLATE III

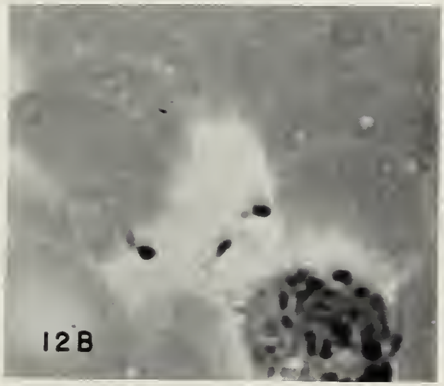
Fig. 8. Male gametocyte slipping out of capsule. This occurs often but not always. 4 flagella have formed and the chromatin is still in 2 thick strands, the pairs arranged end to end (*vivax*). × 4050+.

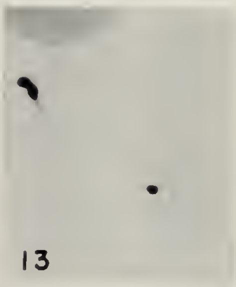
Fig. 9. An abnormal male gamete included to show double nature of the cytoplasmic element and also to show that the male gamete often contains "blobs" of cytoplasm either pushed ahead of the chromatin or pulled after it (*vivax*). × 4050+.

Fig. 10, A and B. Male gametocyte slipping out of capsule. 4 pairs of chromosomes are clearly seen with heterotypic pair on the left. Only one of these pairs will enter each male gamete but each of these masses may represent more than 1 pair of chromosomes.

11 A

12 A





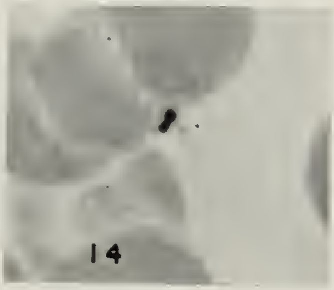


Plate IV

Fig. 11. The chromatin element is joining the cytoplasmic element in 2 sperm. Note the 2 paired masses that have not entered cytoplasmic elements as yet. × 4050+.

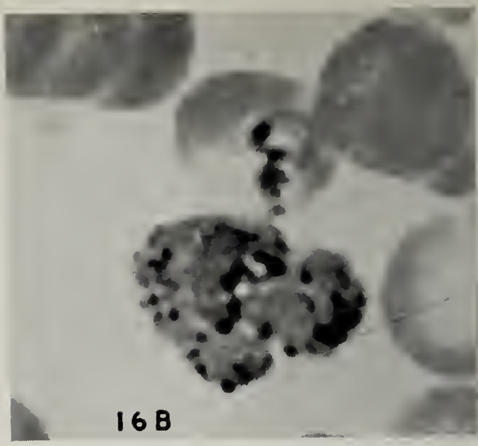
Fig. 12. Male gametes (sperm) that have just separated from the old gametocyte. The head appears solid at this stage. The lighter portion behind the head is a "blob" of cytoplasm. × 4050+.

Fig. 13. The chromatin mass in the head separated into two masses almost immediately (*vivax*). × 4050+.

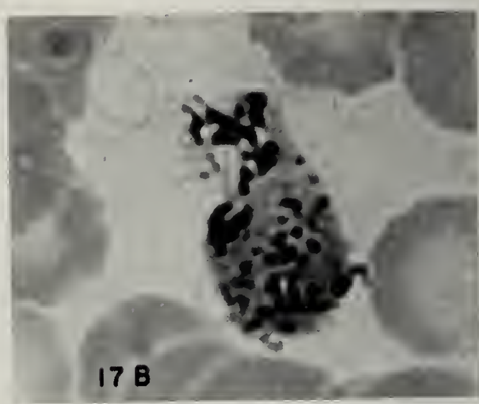
Fig. 14. A loop is formed at the end of the sperm and the two masses move toward the anterior end (*vivax*). × 4050+.

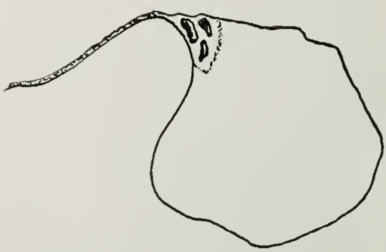
Fig. 15. Mature sperm is now ready to fertilize the female gamete (*vivax*). × 4050+.

16 A



17 A





18 A

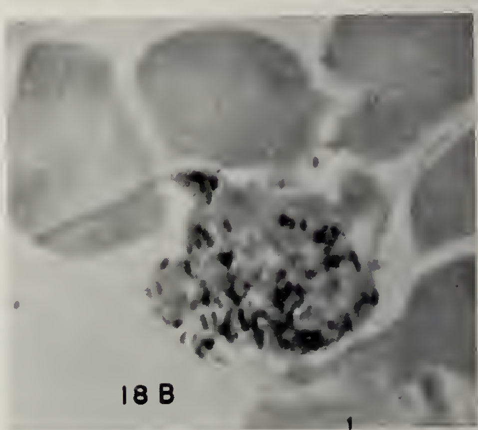


PLATE V

Fig. 16, A and B. When mature, the female gamete puts out a cone and sperm comes to rest against it. In Fig. 16, the sperm approaching the cone is in the stage shown in Fig. 14 (*vivax*). X 4050+.

Fig. 17, A and B. Mature sperm fusing with female gamete. Note that the 2 chromatin masses are entering the female gamete (vivax). X 4050+.

Fig. 18, A and B. Fertilization. The 2 gametes have fused but the chromatin picture is not clear in this cell (*vivax*). × 4050+.

Figure 12. Usually there is a lighter "blob" of cytoplasm that has been dragged into the cytoplasmic element by the chromatin. Almost immediately, the chromatin separates into two masses and a loop is formed at the end of the sperm which is now mature. The 2 masses move toward the end of the loop, Figures 14 and 15, and the sperm is now mature.

Sterile Sperm

All of the slides examined that have shown fair gametocyte counts also have shown that many of the flagella break off from the male gametocyte without the chromatin element and are, therefore, sterile (Fig. 7). In the Columbia Laboratory, it was noted that in certain cases showing very high gametocyte counts of *falciparum*, the mosquitoes feeding on these patients either developed light or no infections. Careful examination of these cases showed that the great majority of the sperm that were free from the gametocytes showed no chromatin elements. At this time we have no explanation as to the stimulus that caused these flagella to break off prematurely. It is quite certain that in both *falciparum* and *vivax*, even where good mosquito infections are obtained, there are many sperm that never receive the chromatin element and are, therefore, sterile.

Fertilization

The mature female gamete puts out a cone to which the sperm adheres, Figures 16 and 17. By this time the 2 chromatin masses have moved to the end of the loop (Figure 17) and union of the male and female gametes begins. Note that the masses entering the female are entirely separated at this time. Each of them may be double but this point is difficult to establish before all of the conditions of the reduction division are known. In the female gamete shown in Figure 16, note the dumbbell chromosomes. In Figure 15, the chromatin situation is confused but even here it is clearly shown that the chromosomes are either dividing or paired (?) and that they are of different appearances. There is definitely one long pair, one medium pair, one small pair and more chromatin which may be, among other things, extruded chromatin or polar bodies. There is a dispute about the existence of polar bodies in *Plasmodium*, some chromatin masses being called by Casini (1936) the "so-called polar globules in the gametocytes of malarial parasites". Work is continuing on the chromatin in the female gametocyte. The chromosomes are of different sizes but the manner of reduction division has not been determined. Since only one paired mass goes into each sperm and since these masses are different from each other, especially as regards the heterotypic pair, one looks for the pre-reduction or reduction in formation of gametes. In some *Sporozoa*, however, there is a post-zygotic division as shown by Dobell (1925) in the coccidium *Aggregata eberthi* where the number of 12 chromosomes is reduced to 6 at the first division of the nucleus of the female gamete after fertilization. The scarcity of material to follow the necessary stages is probably due to the fact that they occur very rapidly.

SUMMARY

Although many critical stages are missing, the contributions of this paper are especially: (1) it is shown that there are definite chromosomes in *Plasmodium*, a point questioned by some authorities; (2) though obscure, there is an orderly sequence of events in the behavior of the chromosomes in the formation of the male gamete; (3) the history of the formation of the male gamete is complete except for determination as to whether each of the 2 chromatin masses represents 2 or 4 chromosomes when it enters the immature sperm; (4) the finding of a heterotypic pair of chromosomes which suggests a possible relation to the determination of sex; (5) the stage of fertilization is shown establishing the fact that 2 and only 2 of the paired chromatin masses enter each sperm cell; it is explained why each of the masses may represent 2 chromosomes; (6) an explanation is given as to why mosquitoes feeding upon certain cases of *P. falciparum* showing high gametocyte counts often become lightly infected or not at all, the reason being that the chromatin element fails to join the cytoplasmic element of the sperm in these cases; the sperm are, therefore, sterile.

RESÚMEN

Auncuando no se conocen muchos estados críticos, las contribuciones de este papel son especialmente: (1) se demuestra que hay definidos cromosomas en *Plasmodium*, un punto cuestionado por algunos autories; (2) aunque oscuro, hay un orden definido en el comportamiento de los cromosomas en la formación del gameto macho; (3) La historia de la formación del gameto macho es completa excepto la determinación de si cada una de las dos masas de cromatina representan dos ocuatro cromosomas cuando éste entra a la esperma inmadura; (4) el hallazgo de un par heterotípico de cromosomas que sugiere una posible relación con la determinación del sexo; (5) el estado de fertilización se demuestra estableciendo el hecho que dos y únicamente dos delas masas cromaticas pareadas entran en cada célula espermática; ésto es explicado de por qué las masas pueden representar dos cromosomas; (6) se dá una explicación de por qué los mosquitos que se alimentan en ciertos casos de *Plasmodium falciparum* mostrando altas cuentas gametocíticas frecuentemente adquieren una infección liviana o no se infectan del todo, la razón es que el elemento cromático falla en unir el elemento citoplasmático de la esperma en estos casos; la esperma es pues, estéril.

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STUDIES ON THE USE OF 2,4-D FOR THE CONTROL OF PLANTS IN A MALARIA CONTROL PROGRAM*

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The production of *Anopheles quadrimaculatus*, the only important vector of malaria in the Southeastern States, is invariably associated with the presence of plants and flotage in the waters of the breeding area. In many situations, the most efficient measures for preventing the propagation of this mosquito are those which eliminate the plants which create favorable conditions for its production. For this reason plant control constitutes one of the major elements in the Tennessee Valley Authority's program of malaria control. This program involves nine major river and sixteen storage reservoirs with a total shoreline of almost 10,000 miles and an area of about 600,000 acres. The major plant control operations are directed at the terrestrial and wetland species which invade the marginal zones of fluctuation, but the control of certain deep-water aquatics is also a highly essential element.

In general, phytocides have had a minor role in the Authority's plant control operations, although in some instances they have been used with considerable success as, for example, in the control of alligator weed on Lake Wilson through the application of an oil herbicide. The recent discovery of the remarkable phytocidal properties of 2,4-D (2,4-dichlorophenoxyacetic acid) and related growth-regulating substances caused the Authority to reappraise the status of phytocides in its plant control operations. During the summer of 1945, small-scale field tests were carried out with 2,4-D and some of its derivatives to find out if these materials might be used to control some of the problem species in the Tennessee Valley. The results were so promising that an intensive program of study was conducted during the 1946 season to determine the relative effectiveness of various materials, formulations, and concentrations and to develop satisfactory methods of application. In addition, certain basic observations were made on the mode of action of the new formagenic phytocides. The present paper presents a summary of these studies and the results obtained.

BASIC OBSERVATIONS

Space will not permit a detailed account of the basic observations on growth-regulating substances. However, a brief summary of certain of the results will be of interest in connection with the control studies.

General Effects of Formagenic Substances on Plants

2,4-D and related substances achieve their effects upon plants through a physiological derangement. This derangement frequently results in abnormal growth

* This paper was presented at the annual meetings of the National Malaria Society in Miami, Florida, 5 November 1946.

forms, for which reason the term "formagenic" is frequently applied to these substances. Since the morphological responses are produced at considerable distances from the point of application, these effects have been termed "telemorphic." In some of the species with which the present studies were concerned the application of 2,4-D resulted in a greatly increased growth rate and the production of unusual forms (see Fig. 1). Among plants so affected were American lotus,[1] giant and lesser ragweeds, horseweed, Spanish needles, white top, and cotton. In other species, such as green ash and buttonball, growth was arrested by the application of 2,4-D. The common expression that 2,4-D causes plants to "grow themselves to death" is therefore not always applicable. In general, 2,4-D was most effective in controlling those species in which it produced the strongest morphological response.

The importance of concentration in relation to the formagenic effects of 2,4-D was demonstrated with lotus seedlings. In Fig. 2 are presented data on lotus seedlings grown for 15 days at 70°F. in concentrations of 2,4-D varying from 0 to 10,000 ppm, 50 fruits being used at each concentration. It will be observed that growth was stimulated at the intermediate concentrations and suppressed at the higher concentrations. Similar observations with vegetative segments of alligator weed gave entirely different results, there being a general suppression of growth at all concentrations. The variation in the results obtained with lotus and alligator weed may be related to the differences in the tolerance of these two plants to 2,4-D, and emphasizes the importance of studying the reactions of individual species.

Another interesting effect of 2,4-D upon plants was the suppression of flowering and fruiting in species such as lotus, buttonball, and cotton. This characteristic action might be used to advantage in controlling certain species which propagate mainly by seeds.

Absorption and Translocation of Formagenic Substances

The destructive effects of 2,4-D and related materials are not dependent upon their direct caustic action but apparently upon the extent to which they are absorbed and their active principle transported to the various parts of the plant. Observations were therefore made on the absorption and translocation of 2,4-D in some of the representative problem species of the Tennessee Valley. An attempt was made to use fluorescent dyes as tracers for following the course of 2,4-D through the plant tissues. The results were unsatisfactory, however, because of the apparent dissociation of the active principle from the dyes, the formagenic effects of the 2,4-D being observed for considerable distances beyond the farthest point at which the dyes could be recovered. The observation of leaf and stem curvatures therefore appeared to be the only satisfactory means available for determining the extent to which the active principle of 2,4-D was transported through the plant tissues.

Information on the upward, downward, and lateral transport of 2,4-D in black willow was obtained by placing single roots or stems in solutions of 2,4-D and observing the subsequent effects on various parts of the plant. The results indicated that the active principle of 2,4-D is readily transported upward, downward, and

[1] For scientific names of plants mentioned in the text, see Tables 5 and 6.

FIG. 1. Some Formagenic Effects of 2,4-D Upon Plants: (A) Abnormally dissected leaves of cotton produced by the methyl ester of 2,4-D; (B) Typical "S" curvature of giant ragweed produced by the sodium salt of 2,4-D; (C) Spring-like coils of lotus petiles produced by the triethanolamine salt of 2,4-D; (D) Narrow pinnatifid leaves of stinkweed produced by the sodium salt of 2,4-D.

laterally in black willow. Further evidence of this was given by the fact that when the apices of single shoots of willow coppice were placed in 2,4-D solutions, typical curvatures appeared in other shoots growing on the same stumps. By the use of girdling techniques it was demonstrated that the active principle of 2,4-D was readily transported through the xylem, but the downward transport was apparently limited to the phloem, except possibly when conditions reversed the flow of the

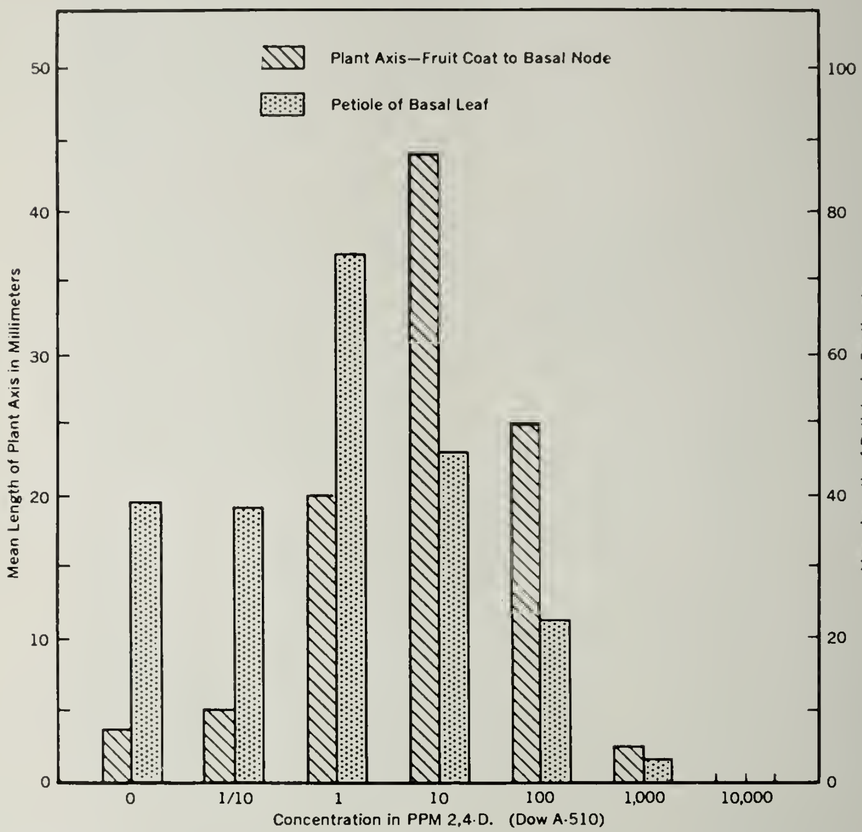

FIG. 2. Effect of Varying Concentrations of 2,4-D Upon the Growth of Petioles and Plant Axes of Lotus Seedlings Grown for 15 Days at a Constant Temperature of Approximately 70° Fahrenheit. Fifty Fruits Were Used at Each Concentration.

transpiration stream. Observations with buttonball, trumpet vine, and green ash indicated that the active principle of 2,4-D was readily transported upward in these plants, but there was no significant downward transport. This may at least partially explain why these species are so much more resistant to 2,4-D treatments than is black willow.

Studies with alligator weed, using the split stem technique, gave evidence that downward transport was also quite limited in this species, thus indicating the desirability of dewatering in order to achieve the most effective control from applications

of 2,4-D. Observations with parrots feather showed no significant upward transport of the active principle of 2,4-D and only a very limited downward transport. The relatively high tolerance of this and other submerged aquatics to 2,4-D is probably related to their reduced vascular system and the resultant limited transport of the formagenic substances when treated with 2,4-D. Dewatering before treatment would therefore appear essential for the most effective control of these species with 2,4-D.

Residual Action of Formagenic Substances

The residual effects of applications of 2,4-D are generally considered to be lost within 8 to 12 weeks, depending upon the condition and type of soil, the amount of rainfall, and the plant species. It was, therefore, interesting to observe residual effects persisting in black willow from one season until the next. During mid-season of 1945, some willow coppice about 18 feet tall was sprayed with an aqueous solution of 2,4-D, resulting in the death of most of the above-ground portions. The following spring new shoots were produced from the trunks just above the soil level; however, these shoots exhibited typical 2,4-D curvatures and subsequently died. No effects of 2,4-D were apparent in the broadleaf herbs growing in the soil around the treated willows. It therefore appears that the active principle of 2,4-D was stored in the dormant vegetative bodies of the willows over the winter months. Similar evidence of overwinter storage was observed with buttonball.

CONTROL STUDIES

Hand Application

During the 1946 season detailed observations on the control effectiveness of hand applications of 2,4-D were made with the following sixteen species (for scientific names, see Tables 5 and 6).

1. Black Willow
2. Burhead
3. Buttonball
4. Cattail
5. Cocklebur
6. Cowlily
7. Giant Cutgrass
8. Giant Ragweed
9. Lizard Tail
10. Lotus
11. Weak Rush
12. Trumpet Vine
13. Water Primrose
14. Dianthera
15. Wild Cotton
16. Woolgrass

In addition to the detailed studies on the above plants, general observations were made on the effect of 2,4-D upon sixty-six other species, making a total of twenty-four woody and fifty-eight herbaceous species for which control data were obtained (see Tables 5 and 6). The test plots were located in the Kentucky, Pickwick, Wilson, and Wheeler Reservoir areas.

The lotus plots were 100 x 100 feet, and for all other species plots 40 x 40 feet were used. Ten sampling quadrats were located at established intervals in the central 20 x 20 feet area of each plot, square-yard wooden frame quadrats being used in lotus and square-foot quadrats in all other species.

Density and cover data were recorded for each quadrat just prior to treatment and at appropriate intervals following treatment. Cover was expressed as percentage of total ground cover as determined by visual appraisal of the individual quadrats from the vertical aspect. Density was expressed for most species as the number of individual plant stems per quadrat as determined by actual counts; however, in the case of cowlily and lotus these counts referred to the flower peduncles or leaf petioles which arise directly from the underground stems (rhizomes). Only living plants or leaves were included in post-treatment cover and density data. An untreated control plot was also checked for each species at each sampling period in order to provide comparisons with normal seasonal changes.

Four species (cocklebur, lotus, lizard tail, and black willow) were selected for detailed comparisons of the following five 2,4-D formulations applied in single treatments at rates of about 1 and 3 lbs. 2,4-D per acre:

Aqueous solution of sodium salt[2]	½% 2,4-D
Aqueous solution of triethanolamine salt[3]	½% 2,4-D
Water emulsion of methyl ester[4]	½% 2,4-D
Water emulsion of methyl ester[4]	5% 2,4-D
Kerosene solution of butyl ester[5]	4% 2,4-D

Duplicate plots for each treatment rate were used. The materials were applied with a compressed air sprayer operated at 40 to 50 pounds pressure. Two types of nozzles were used: a standard whirl disc type for the lower concentrations, and a mist type[6] for the higher concentrations. The relatively high concentration sprays were used in order to eliminate the handling of unnecessary volumes of diluent.

In addition to spray plots treated with the above materials, three lotus plots were treated with a 10 per cent 2,4-D dust applied by a hand rotary duster at rates of 2.5, 5, and 10 pounds of 2,4-D per acre.

The remaining twelve species of plants for which detailed studies were made were treated only with the sodium salt applied as a ½ per cent aqueous solution of 2,4-D applied in single treatments at rates of 1 and 3 pounds 2,4-D per acre. Duplicate plots were not used for these species.

Results of the comparative tests with cocklebur, lotus, black willow, and lizard tail are summarized in Table 1. It will be noted that cocklebur and lotus were both highly susceptible to 2,4-D, effective control of both of these species being obtained with all of the formulations at the one pound per acre dosage. In contrast, lizard tail was highly resistant, although significant reductions in both cover and density were indicated by comparison with the untreated check plots. Black willow was significantly less susceptible than cocklebur and lotus; however, a complete appraisal of the control of willow cannot be made until next season because of the previously discussed residual effects of 2,4-D which have been observed in this species.

[2] Dow Chemical Co., Midland, Mich., and E. O. Link Co., Inc., Montclair, N. J.
[3] U. S. Rubber Co., New York 20, N. Y.
[4] Dow Chemical Co., Midland, Mich
[5] Sherwin-Williams Co., Cleveland, Ohio.
[6] Spraying Systems Co., No. 1/4 LN2

In general, there were no great differences in the control obtained with the different formulations; however, there were some indications that the salts were somewhat

TABLE 1

*Comparative Effectiveness of Hand Applications of Various Formulations of 2,4-D Applied to Cocklebur, Lotus, Black Willow, and Lizard Tail, 1946 Season**

SPECIES AND FORMULATIONS	PER CENT CHANGES FOLLOWING TREATMENT†			
	1 lb. 2,4-D per acre		3 lbs. 2,4-D per acre	
	Density	Cover	Density	Cover
1. Cocklebur				
½% Sodium Salt	−97	−99	−100	−100
½% Triethanolamine Salt	−88	−96	−99	−99
½% Methyl Ester	−82	−92	−99	−99
5% Methyl Ester	−64	−76	−99	−99
4% Butyl Ester	−72	−88	−97	−99
Untreated check	−20	+3	−20	+3
2. Lotus				
½% Sodium Salt	−74	−82	−84	−90
½% Triethanolamine Salt	−79	−84	−90	−91
½% Methyl Ester	−97	−97	−99	−99
5% Methyl Ester	−92	−93	−100	−100
4% Butyl Ester	−91	−89	−100	−100
Untreated check	+5	0	+5	0
3. Black Willow				
½% Sodium Salt	−3	−73	−18	−97
½% Triethanolamine Salt	−20	−97	−56	−96
½% Methyl Ester	−22	−82	−58	−98
5% Methyl Ester	−24	−76	−31	−86
4% Butyl Ester	−18	−72	−37	−92
Untreated check	0	+45	0	+45
4. Lizard Tail				
½% Sodium Salt	+18	+33	−2	+65
½% Triethanolamine Salt	−12	+37	−1	+40
½% Methyl Ester	−16	−36	−15	−35
5% Methyl Ester	+100	+91	−8	−37
4% Butyl Ester	+118	+141	+63	+22
Untreated check	+227	+750	+227	+750

* Cocklebur treated July 25–29 and sampled 3 weeks later. Lotus treated July 11–15 and sampled 8 weeks later. Black willow treated June 24–30 and sampled 7 weeks later. Lizard Tail treated July 16 and sampled 8 weeks later.

All density and cover averages are given in the nearest whole per cent except that all averages between 99 and 100 per cent are listed as 99 in order to show all instances of incomplete control.

† All materials applied at 1 and 3 lbs. 2,4-D per acre except butyl ester which was applied at 0 8 and 2.4 lbs. 2,4-D per acre.

more effective against cocklebur, while the esters were more effective against lotus. There was also some evidence that the methyl ester might be more effective against lizard tail than the other materials.

It is interesting to compare the results obtained with the ½ per cent and 5 per cent emulsions of the methyl ester applied at the rate of 1 pound 2,4-D per acre. For every one of the four species, a more effective control was obtained with the ½ per cent emulsion than with the 5 per cent emulsion. These results reflect the unevenness of hand distribution and indicate that for hand application methods the spray concentration should not be more than ½ per cent in order to provide a sufficient volume of spray for adequate coverage. Drift of material away from the treatment areas and evaporation of solvent may also have been factors in the reduced control obtained on the higher concentration plots since these were treated with a finer mist-type spray.

The results of the applications of 2,4-D dust to lotus are given in Table 2. It will be observed that at equivalent dosages the dusts did not give as satisfactory results as the sprays, although highly effective controls were obtained with the 5 and 10 pound rates of application.

TABLE 2

Effectiveness of 10 Per cent 2,4-D Dusts Applied to Lotus Plots Treated July 23 and Sampled 30 Days Later*

PER CENT CHANGES AT INDICATED TREATMENT RATES OF 2,4-D							
2-1/2 lbs. per acre		5 lbs. per acre		10 lbs. per acre		Untreated	
Density	Cover	Density	Cover	Density	Cover	Density	Cover
−84	−70	−98	−98	−98	−97	+5	0

* Dow 650, Dow Chemical Company, Midland, Michigan.

The results obtained from the application of the sodium salt of 2,4-D to twelve species are summarized in Table 3. There was a wide variation in the control achieved for the different species. Burhead, giant ragweed, trumpet vine, water primrose, and wild cotton were highly susceptible to 2,4-D, at least when dewatered, and were effectively controlled at an application rate of 1 pound per acre. Cowlily, weak rush, and dianthera were intermediate in susceptibility. Buttonball, cattail, giant cutgrass, and woolgrass were highly resistant to 2,4-D, and little or no reduction in these species was achieved even at dosages of 3 lbs. per acre. It should be pointed out, however, that a final appraisal of the results obtained with the latter perennial species cannot be made until next year.

Application by Ground and Boat Power Equipment

Growth-regulating substances were dispersed from a command car equipped with a portable spraying unit. This spraying unit consisted of a Bean "Junior Duplex Pump" driven by a Cushman two horsepower "Huskey" air-cooled engine equipped with a portable spray boom and a Bean "Spray Master Deluxe" spray gun. The boom gave an effective swath width of twenty feet and the spray gun was equipped with 100 feet of hose in order to reach additional areas not accessible for spraying with boom. More recently a larger capacity sprayer has been developed by the engi-

TABLE 3

Effectiveness of Hand Applications of a ½ Per cent Solution of the Sodium Salt of 2,4-D Applied at Rates of 1 and 3 Pounds 2,4-D per Acre to Twelve Species of Plants, 1946 Season

SPECIES	PER CENT CHANGES FOLLOWING TREATMENT*					
	1 lb. 2,4-D per acre		3 lbs. 2,4-D per acre		Untreated	
	Density	Cover	Density	Cover	Density	Cover
Burhead	−99	−99	−100	−100	0	+94
Buttonball	−6	+7	−33	−51	−10	+86
Cattail	+51	+76	+58	+21	+26	+40
Cowlily	−46	−52	−33	−43	−25	−24
Giant Cutgrass	+8	+45	+25	+56	+4	+43
Giant Ragweed	−98	−94	−100	−100	−35	−30
Weak Rush	−52	−91	−65	−95	+14	−22
Trumpet Vine	−94	−98	−100	−100	−17	+86
Water Primrose	−7	−99	+15	−99	−46	−30
Dianthera	+31	−30	−25	−82	+27	+33
Wild Cotton	−59	−81	−87	−98	+100	+325
Woolgrass	+15	+4	+22	−4	+29	+2

* Plots treated June 26 to July 10 and sampled 41 to 56 days after treatment. All density and cover averages are given in the nearest whole per cent except that all averages between 99 and 100 are listed as 99 in order to show all instances of incomplete control.

FIG. 3. Command car equipped with dual tires, spray boom, and hand spray gun for application of 2,4-D sprays.

neering staff, the length of the boom has been increased to give an effective swath width of about 30 ft., and dual tires have been added to provide improved locomotion over soft ground (Fig. 3). The boom was equipped with six nozzles (Spraying

Systems Company No. 8002) and the spray was discharged from both the boom and spray gun at approximately 200 psi. The same portable spray unit was mounted in a boat and sprays applied either with the spray gun or with a perforated T boom.

Growth-regulating substances were applied with this portable unit to a number of shoreline testing plots in Kentucky, Pickwick, Wheeler, Guntersville, and Norris Reservoirs. The sprays were applied mainly at rates of one and three pounds of 2,4-D per acre at concentrations of $\frac{1}{10}$, $\frac{1}{2}$, and $1\frac{1}{2}$% 2,4-D, and the volumes required for approximately 3 lbs. of 2,4-D per acre were about 375 gals., 75 gals., and 25 gals., respectively. The sodium salt, triethanolamine salt, methyl ester, and butyl ester of 2,4-D were all applied successfully with this type of equipment.

In general, the results obtained with the power equipment were in close agreement with those from smaller experimental areas treated by hand. Lotus, willows, goldenrod, wild cotton, ragweeds, and cocklebur proved to be quite susceptible to the sprays, whereas giant cutgrass, cattail, and woolgrass proved to be quite resistant. The methyl ester showed some promise for the control of cattail, particularly when applied to the younger plants. Giant cutgrass survived spraying with the butyl ester in kerosene at a rate of 3 lbs. of 2,4-D and 75 gallons of kerosene per acre. Dianthera appeared to be controlled readily if sprayed when dewatered with the sodium salt of 2,4-D (0.1%) applied at a rate of 3 lbs. 2,4-D per acre.

Airplane Application

The low effective dosages of 2,4-D phytocides and the availability of high concentrate materials make it feasible to use airplanes for their distribution. Accordingly, tests were carried out during the 1946 season on the airplane application of 2,4-D dusts and sprays, using lotus as the test plant.

Dust applications were made with one of the Authority's standard Model 4-DX Stearman dusters equipped with 450 h.p. Pratt and Whitney engine (Kruse' et al., 1944). The dust formulation used was 10 per cent 2,4-D in pyrax. The dust was discharged at the rate of 12 lbs. per acre on a 100-foot swath basis, which was equivalent to 1.2 lbs. 2,4-D per acre. The specific gravity of the dust was 2.75 and it weighed approximately 50 lbs. per cubic foot. The mass median diameter of the particles was estimated to be about 36 microns.

The 2,4-D sprays were applied with a Vultee BT-13A (Fig. 4) powered with the same type of 450 h.p. engine as used on the Stearman dusters. During spraying operations this ship was flown at a speed of 120 miles per hour, giving a coverage of 24 acres per minute on a 100-foot swath basis as compared with 21 acres per minute for the Stearman flying at 105 mph. The spraying equipment consisted of an electric pump taking the solution from one of the wing tanks and pumping it at 40 pounds pressure through internal feed lines to five discharge nozzles,[7] one of which was located at each wing tip, one on each stabilizer tip, and one on the tail cone. The rate of discharge was 3.5 gallons per minute or about 0.6 quart per acre on a 100-foot swath basis. The mass median diameter of the droplets produced was about 400 microns with an average range in size of 75 to 500 microns in diameter.

[7] No. 1/4 T 8010, Spraying Systems Co., Chicago, Ill.

In Fig. 5 are presented representative swath recoveries for the dusts and sprays as determined by the examination of glass slides placed at 20-foot intervals across the central 200 feet of the flight swaths. It will be noted that there was a much higher recovery with the sprays than with the dusts, about 66 per cent of the discharged material being recovered in the center 50 feet of the spray swath as compared with only 30 per cent for the dust swath. With a discharge rate of about 1 lb. 2,4-D per acre per 100-foot swath, the average number of dust particles per square inch recovered in the central 50 feet of the dusting swath was 135. The average number of droplets per square inch recovered in the central 50 feet of the spray swaths at a similar discharge rate was 25 for 72 per cent butyl ester, 50 for 40 per cent

FIG. 4. View of Vultee BT-13 equipped for applying sprays. Arrows indicate discharge nozzles at wing tips and stabilizer tips and spray boom beneath tail cone. Note also venturi exhaust generator for distributing DDT thermal aerosols.

butyl ester, 97 for 27 per cent triethanolamine salt, and 137 for the 20 per cent methyl ester. The number of droplets per square inch was, of course, proportional to the total volume of discharge, but even with the most concentrated materials an adequate coverage was obtained.

A general summary of the results obtained from six tests on the airplane distribution of 2,4-D sprays for the control of lotus is given in Table 4. The results obtained from the application of these high concentrate materials were highly satisfactory. For example, the single flight tests with 72 per cent butyl ester gave essentially a 100 per cent kill over a 112 ft. swath at an average dosage of less than a pound of 2,4-D and about 1 pint of liquid per acre. As will be observed from the swath recovery rates given in Fig. 5, this indicates effective control of lotus at the edges of the swath with dosages as low as 0.1 to 0.2 lb. 2,4-D per acre. With the

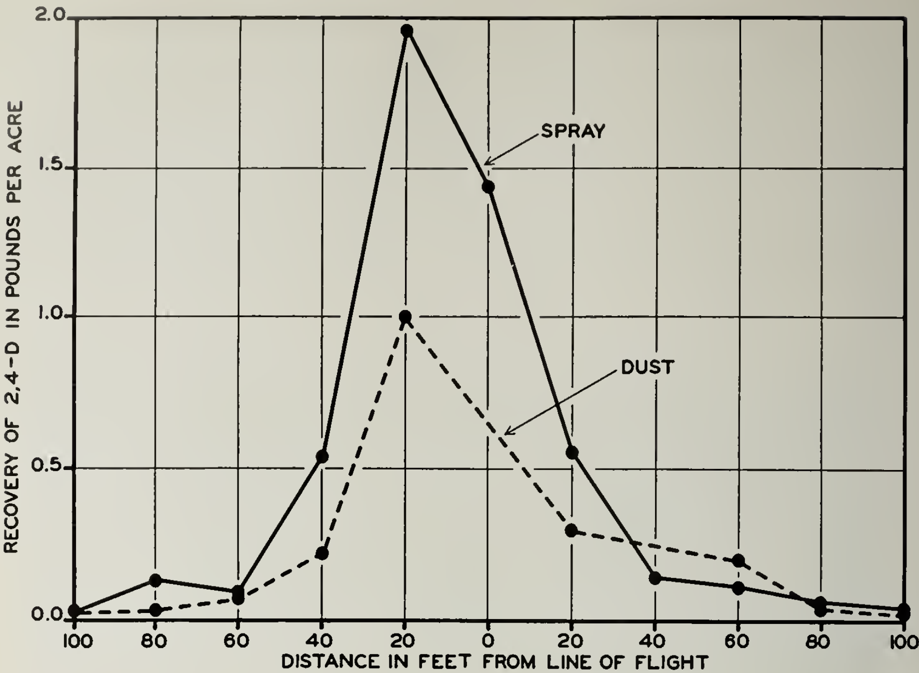

FIG. 5. Swath Recoveries for 2,4-D Dusts and Sprays Distributed by Airplane at Similar Rates of Application. (Spray was butyl ester 72% 2,4-D Applied at 1.0 lb. 2,4-D per acre per 100 ft. swath. Dust was 10% 2,4-D applied at 1.2 lbs. per acre).

TABLE 4

Effectiveness of Airplane Applications of Various 2,4-D Formulations to Lotus, 1946 Season

NUMBER OF FLIGHTS OVER SWATH	FORMULATION	DISCHARGE RATES PER ACRE/100 FT. SWATH		SWATH WIDTHS		AVERAGE DOSAGE PER ACRE FOR AREA OF 95–100% KILL	
		Gals.	Lbs. 2,4-D	95–100% Kill	Partial control	Lbs. 2,4-D	Gals. liquid
7	Methyl Ester, 20% 2,4-D	1.05	1.7	273 ft.	92 ft.	0.5	0.38
5	Tri-Salt, 27% 2,4-D	0.75	1.9	97 ft.	74 ft.	2.0	0.77
8	Tri-Salt, 27% 2,4-D	1.20	3.0	100% kill 35 acres	—	3.0	1.2
3	Butyl Ester, 40% 2,4-D (in kerosene)	0.45	1.4	87 ft.	97 ft.	1.6	0.52
2	Butyl Ester, 72% 2,4-D	0.30	2.1	278 ft.	80 ft.	0.8	0.11
1	Butyl Ester, 72% 2,4-D	0.15	1.0	112 ft.	20 ft.	0.9	0.13

lower concentration materials, such as the 20 per cent methyl ester, it was necessary to make more flights in order to apply an equivalent dosage. In making these

repeated flights over the same area it was not possible to stay on exactly the same flight line each time which probably explains why the effective swath width per pound of 2,4-D discharge was greater on these flights. Because of the greatly increased payload and the highly effective results obtained with the high concentrate materials, they would appear more desirable for use in airplane spraying than the lower concentration materials. From the one test run with the triethanolamine salt it would appear that this material is not as effective in airplane application as are the methyl and butyl esters. However, even this material will give highly effective control if applied in sufficient dosage as was indicated by the complete eradication of 35 acres of lotus with this material in test No. 3.

Ten per cent 2,4-D dust was applied to a 30-acre tract in the Kentucky Reservoir area infested with lotus. The entire slough was treated on June 24 at a rate of 2.5 lbs. 2,4-D per acre and a portion was retreated at the same rate on July 26. A reduction in cover of about 60 per cent was obtained in the single treatment area and a reduction of about 90 per cent was obtained in the area receiving two treatments. This indicates that at equivalent dosages the dusts were not as effective as sprays in controlling lotus, which is in agreement with the hand treatment data. Because of this reduced effectiveness and decreased payloads, airplane dusting is not considered as satisfactory as airplane spraying as a means of applying 2,4-D to lotus.

From the standpoint of both cost and effectiveness, it appears that the airplane is the most satisfactory means of distributing 2,4-D for the control of susceptible plants such as lotus on an area basis. This method eliminates the spotty coverage which is characteristic of hand and ground application methods and therefore gives effective control at lower dosages. Also, engineering cost data indicate that the total cost per acre of applying 2,4-D by airplane will be only about one-fourth to one-sixth the cost of applying it by hand or power spray units. It is obvious, of course, that for small "patch" treatments, areas inaccessible by plane, etc., ground and hand methods of application will have to be used.

Airplane distribution tests have also been carried out with black willow, but it will be impossible to appraise the effectiveness of these treatments until the next growing season.

EFFECT OF 2,4-D ON CROP PLANTS

During the course of the studies, it was found that cotton was extremely susceptible to 2,4-D sprays and was affected adversely due to drift of small quantities of the spray a few hundred feet from the site of application. Corn and sorghum showed no effects from 2,4-D drift which affected cotton, and these monocots are considered to be relatively resistant to this type of spray. *Lespedeza* sp. survived on plots treated with the sodium salt of 2,4-D at rates up to 3 pounds per acre. Johnson grass was also found to be very resistant to 2,4-D sprays.

It should be emphasized that great caution must be exercised in the use of 2,4-D in the vicinity of susceptible crops such as cotton, particularly in the case of airplane application.

GENERAL DISCUSSIONS

From the detailed observations on sixteen species of plants, the general observations on sixty-six additional species, and the results of ground, boat, and airplane treatments, a general classification has been made of the susceptibility to 2,4-D of the 24 woody and 58 herbaceous species for which control data were obtained. This

TABLE 5

A Tentative Table of the Relative Susceptibility of Woody Plants to 2,4-D Based on Observations Carried Out in the Tennessee Valley During the Seasons of 1945–1946

High Susceptibility	
Black locust	*Robinia pseudo-acacia* L.
Black willow	*Salix nigra* Marsh.
Cottonwood	*Populus deltoides* Marsh.
Hackberry	*Celtis mississippiensis* Bosc.
Japanese honeysuckle	*Lonicera japonica* Thunb.
Sassafras	*Sassafras officinale* Nees. & Eberm.
Sycamore	*Platanus occidentalis* L.
Medium Susceptibility	
Blackberry	*Rubus* sp.
Florida vine	*Brunnichia cirrhosa* Banks.
Green ash	*Fraxinus pennsylvanica* March. var. *lanceolata* (Borkh.) Sarg.
Overcup oak	*Quercus lyrata* Walt.
Pepper vine	*Ampelopsis arborea* (L.) Rusby.
Persimmon	*Diospyros virginiana* L.
Poison ivy	*Rhus toxicodendron* L.
Sweet gum	*Liquidambar styraciflua* L.
Trumpet vine	*Campsis radicans* Seerman.
Tupelo gum	*Nyssa aquatica* L.
Virginia creeper	*Parthenocissus quinquefolia* (L.) Planch.
Low Susceptibility	
Bald cypress	*Taxodium distichum* (L.) Rich.
Buttonball	*Cephalanthus occidentalis* L.
False indigo	*Amorpha fruticosa* L.
Red cedar	*Juniperus virginiana* L.
Scrub pine	*Pinus virginiana* Mill.
Silver maple	*Acer saccharinum* L.

classificiation is given in Tables 5 and 6. It should be pointed out, of course, that this classification is only tentative since variations in rainfall, soil type, soil moisture, and other factors are known to have a significant effect upon the reaction of plants to 2,4-D, and several seasons' observations will therefore be required before fully reliable classifications can be made. However, it is evident from even this one season's results that certain species, such as ragweeds, cocklebur, lotus, etc., are

highly susceptible and that applications of 2,4-D can be relied upon to give effective control of these plants. It is also evident that certain species, such as buttonball,

TABLE 6

A Tentative Table of the Relative Susceptibility of Herbaceous Plants to 2,4-D Based on Observations Carried Out in the Tennessee Valley During the Seasons of 1945–1946

High Susceptibility	
American lotus	*Nelumbo pentapetala* Walt.
Ammannia	*Ammannia coccinea* Rottb.
Aster	*Aster* sp.
Buckhorn plantain	*Plantago lanceolata* L.
Burhead	*Echinodorus radicans* (Nutt.) Engelm.
Cocklebur	*Xanthium americanum* Walt.
Cotton	*Gossypium* sp.
Dandelion	*Taraxacum officinale* Weber.
Giant ragweed	*Ambrosia trifida* L.
Goldenrod	*Solidago altissima* Nutt.
Hairy water primrose	*Jussiaea grandiflora* Michx.
Horseweed	*Erigeron canadensis* L.
Jimson weed	*Datura stramonium* L.
Lesser ragweed	*Ambrosia elatior* L.
Parrots feather	*Myriophyllum brasiliense* Camb.
Spanish needles	*Bidens aristosa* (Michx.) Britt.
Spiny waterleaf	*Hydrolea quadrivalvis* Walt.
Square-stem spikerush	*Eleocharis quadrangulata* (Michx.) R. & S.
Stinkweed	*Pluchea* sp.
Swamp loosestrife	*Decodon verticillatus* (L.) Ell.
Toothcup	*Rotala ramosior* (L.) Koehne.
Umbrella sedge	*Cyperus pseudovegetus* Steud.
Water primrose	*Jussiaea diffusa* Forsk.
Water willow	*Ludvigia alternifolia* L.
Wild cotton	*Hibiscus militaris* Cav.
Wild cotton	*Hibiscus moscheutos* L.
Medium Susceptibility	
Alligator weed	*Alternanthera philoxeroides* (Mart.) Griseb.
Buttonweed	*Diodia virginiana* L.
Cowlily	*Nuphar advena* Ait.
Dianthera	*Justicia americana* L. (Vahl.)
Dock	*Rumex verticillatus* L.
Giant smartweed	*Polygonum coccineum* Muhl.
Green arum	*Peltandra virginica* (L.) Kunth.
Lespedeza	*Lespedeza* sp.
Nut grass	*Cyperus rotundus* L.
Pennywort	*Hydrocotyle* sp. (Tourne.) L.
Smartweed	*Polygonum hydropiperoides* Michx.
Verbesina	*Verbesina alba* L.
Water purslane	*Ludvigia palustris* (L.) Ell.
Weak-rush	*Juncus effusus* L.
White waterlily	*Nymphaea odorata* Ait.

TABLE 6—*Concluded*

Low Susceptibility	
Beaked-rush	*Rhynchospora corniculata* (Lam.) Gray
Bladderwort	*Utricularia gibba* L.
Broomsedge	*Andropogon virginicus* L.
Bur-reed	*Sparganium americanum* Nutt.
Cattail	*Typha latifolia* L.
Duck potato	*Sagittaria latifolia* Willd.
False-nettle	*Boehmeria cylindrica* L. (Sw.)
Giant cutgrass	*Zizaniopsis miliacea* (Michx.) Doell. & Aschers.
Hornwort	*Ceratophyllum demersum* L.
Johnson grass	*Sorghum halepense* L. (Pers.)
Lizard tail	*Saururus cernuus* L.
Panic grass	*Panicum agrostoides* Spreng.
Rice cutgrass	*Leersia oryzoides* (L.) Swartz.
Water milfoil	*Myriophyllum heterophyllum* Michx.
Wild millet	*Echinochloa crus-galli* (L.) Beauv.
Wild tomato	*Solanum carolinense* L.
Woolgrass	*Scirpus cyperinus* (L.) Kunth.

cattail, woolgrass, and lizard tail are quite resistant to 2,4-D and special formulations and techniques will have to be developed if 2,4-D is to be used effectively against these plants. There seems little possibility that 2,4-D may ever be used to control the grasses because of their high tolerance to it, but plants of this type are relatively unimportant in the Authority's malaria control program.

The work of the past two seasons indicates that many of the plants which create major malaria control problems in the Authority's reservoirs can be more effectively and economically controlled through the use of 2,4-D than by mechanical means. Among these susceptible species are most of the terrestrial stiff-stemmed annuals, lotus, and black willow, and it seems reasonably certain that 2,4-D will find a place in future malaria control programs for use in controlling these plants.

SUMMARY AND CONCLUSIONS

During the seasons of 1945 and 1946 investigations were carried out on the use of 2,4-D (2,4-dichlorophenoxyacetic acid) for the control of plants which create malaria control problems in the reservoirs of the Tennessee Valley Authority. The investigations involved detailed studies with sixteen plant species and general observations with sixty-six additional species, comprising a total of twenty-four woody and fifty-eight herbaceous plants. Certain basic studies were also made on the general effects of 2,4-D and related substances on plants and on the extent of absorption and translocation and residual action in various species.

Materials tested included 2,4-D dusts and the sodium salt, triethanolamine salt, methyl ester, and butyl ester of 2,4-D. The dusts were applied in 10 per cent 2,4-D concentrations at treatment rates of 2.5 to 10 lbs. 2,4-D per acre in hand applications and 2.5 to 5 lbs. per acre in airplane applications. Sprays were applied at dosages of 1 to 3 lbs. per acre in concentrations of 0.5 to 5% 2,4-D in hand treat-

ments, 0.1 to 1.5 % in applications by ground and boat power equipment, and 20 to 72% in airplane treatment. Compressed air sprayers were used for the hand applications and a portable power sprayer for boat application and ground application with a special command car spray rig. Airplane dusting and spraying was by means of the Authority's standard Stearman and Vultee larviciding units.

Certain species appeared to be highly susceptible to 2,4-D and could be effectively controlled at dosages of 1 lb. 2,4-D per acre; these included ragweeds, cocklebur, and other stiff-stemmed terrestrials as well as lotus and black willow. Other species were highly resistant and difficult or impossible to control with a single treatment of 2,4-D; these included the grasses, cattail, lizard tail, woolgrass, and buttonball. Still other species were intermediate in susceptibility, and these included alligator weed, cowlily, weak rush, and dianthera. The eighty-two species studied were tentatively classified in the above three groups (high, intermediate, and low susceptibiltity). There was little evidence that any of the materials tested gave superior control, with the possible exception that somewhat better results were obtained with the sodium salt on cocklebur, with the methyl and butyl esters on lotus, and with the methyl ester on lizard tail.

On the basis of the results obtained it is believed that the use of 2,4-D will provide a more effective and economical means of controlling certain problem species such as lotus, black willow, and most of the stiff-stemmed terrestrials. Airplane application appears to be the most satisfactory method for area treatment of species such as lotus, with ground, boat, and hand application being most suitable for smaller patch treatments. Great caution should be exercised in the use of 2,4-D in the vicinity of highly susceptible crops such as cotton.

RESÚMEN Y CONCLUSIONES

Durante las estaciones de 1.945 y 1.946 se llevaron a cabo investigaciones sobre el uso del 2,4D(2,4-diclorophen oxyacetic acid) para el control de plantas que crean problemas de control de Malaria en los reservorios de la Autoridad del Valle del Tennessee. Las investigaciones constan de estudios detallados con 16 especies de plantas y observaciones generales con 66 especies adicionales, que comprenden un total de 24 plantas tipo arbusto y 48 plantas herbáceas. Ciertos estudios básicos fueron hechos sobre los efectos generales del 2,4-D y substancias parecidas en plantas y sobre la cantidad de absorción y traslocación y acción residual en varias especies.

Los materiales ensayados incluyen 2,4-D en forma de polvo y la sal sódica, sal trietanolamina, ester metílico, ester butílico de 2,4-D. El polvo fué aplicado en concentraciones de 2,4-D al 10 por ciento y las ratas de tratamiento de 2.5 a 10 libras, de 2,4-D por acre usando aplicaciones manuales y 2.5 a 5 libras por acre usando aplicaciones por intermedio de aviones. El rociamiento fué aplicado a dosis de 1 a 3 libras por acre en concentraciones de 0.5 a 5% de 2,4-D en tratamientos manuales y 0.1 a 1.5% en aplicaciones con equipo de tierra o con equipo de botes, y del 20 al 72 por ciento en tratamiento por aviones. Se emplearon roceadores de aire comprimido para las aplicaciones de mano y un roceador de motor para la aplicación por bote y la aplicación desde la tierra, montadas en una camioneta especialmente acondicionada.

La aplicación de polvo y el rociamiento por aeroplano se hizo por medio del avión "Standar Steraman" y las unidades larvicidas "Vultee".

Ciertas especies aparecieron ser muy altamente susceptibles al 2,4-D y pudieron ser efectivamente controladas a la dosis de una libra de 2,4-D por acre; estas incluyen "ragwoods", cocklebur y otras plantas terrestres de raíz dura y también el "lotus" y el sauce "black willow". Otras especies fueron altamente resistentes y difíciles o imposibles de controlar con un simple tratamiento de 2,4-D; éstos incluyen los pastos, cattail, lizard tail woolgrass y buttonball. Otras especies fueron intermediarias en susceptibilidad y éstas incluyen alligator weed, cowlily, weak rush y dianthera. Las 82 especies estudiadas fueron tentativamente clasificadas en los tres grupos (alta, intermedia, y baja susceptibilidad). Se encontró poca evidencia de que alguno de los materiales ensayados diera un control superior, con la posible excepción de los resultados talvéz un poco mejores que se obtuvieron con la sal de sodio en el cocklebur, con el esteres methyl de butyl en el lotus y con el ester de metyl en el lizar tail.

Sobre la base de los resultados obtenidos se crée que el uso de 2,4-D dá método más efectivo y económico de control al tratar ciertos problemas de especies como el lotus, el black willow y muchas de las terrestres de raíz dura. La aplicación por avión parecer ser el método más satisfactorio para el tratamiento de las especies como el lotus, con equipo de tierra, el bote y aplicaciones de mano son las mejores para los tratamientos en pequeñas áreas. Hay qué tener mucho cuidado al usar 2,4-D cuando en la vecindad existen consechas buenas, como el algodón.

ACKNOWLEDGMENTS

Grateful acknowledgment is made of the many individuals who participated in the planning and conducting of these studies. Mr. C. C. Kiker and the following members of the Engineering Staff provided facilities, assistance, and advice for the carrying out of field tests: Robert Sparkman, E. H. Givhan, F. E. Gartrell, C. D. Fairer, and A. H. Johnson. The following individuals also provided much helpful information and advice: Professor Wm. T. Penfound, Miss Margaret Rice, Dr. Paul Denson, Mr. L. W. Kephart and Staff, and Mr. Wesley B. Reeves. Much credit for the success of the field tests should go to Nicholas Drahos and Arthur Morris for their enthusiastic and tireless assistance during the summer of 1946. The data on particle size and swath distribution were compiled by Mr. C. W. Kruse', who also provided much helpful advice during the course of the studies. Airplane treatments were ably carried out by H. B. Seaton and C. E. Bradford. Jeanette Brown and Mary Ella Voorhies tabulated the data and typed and proofread the manuscript. Most other members of the Biology and Engineering Staffs provided assistance in various phases of the work. Representatives of several manufacturing companies gave helpful cooperation, particularly the Dow Chemical Company and the Sherwin Williams Company.

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ENTOMOLOGICAL EVALUATIONS OF RESULTS OF RESIDUAL DDT SPRAYING DURING 1946

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During the past season the Communicable Disease Center of the U. S. Public Health Service in cooperation with state health departments in the Southern States has continued and expanded the Extended Program for malaria control which was begun during 1945. As was discussed at the meetings of this Society last year, this program consists principally of the spraying of DDT in houses to control malaria in the more important endemic areas of the South and to meet the threat of increased malaria transmission occasioned by the return of service personnel who contracted the disease overseas. During 1945 the houses and privies on some 400,000 premises were given one or more treatments with DDT emulsion applied at the rate of 100 mg. of DDT per square foot. During the 1946 season, over 750,000 houses in 266 counties of 13 states were treated with a similar number of residual applications of DDT. In 1946, however, the DDT application rate was increased to 200 mg. per square foot in an effort to secure a longer lasting residual effect and thereby decrease the frequency of application.

In the preceding paper by Dr. Link, available data on the effect of the DDT residual spray in reducing malaria have been presented and discussed. It is the purpose of this paper to present some of the results of the work as measured by entomological criteria. These measurements were of two types:

1. A small percentage of houses selected at random in sprayed areas was inspected to determine the results of the spray in keeping houses free of resting *Anopheles quadrimaculatus*. At the time of each house inspection, the number of *quadrimaculatus* in an adjacent favorable resting place also was determined to obtain a general index to mosquito abundance on the premises. Barns were used principally for the latter purpose. A number of similar house and outbuilding examinations also were made in unsprayed areas for comparison.

2. Precipitin tests were made of blood from engorged specimens of *quadrimaculatus* collected from sprayed and unsprayed areas in order to determine the effect of the spray in reducing the numbers of mosquitoes which feed on man and persist about premises. This method of evaluating results is based on the supposition that *quadrimaculatus* feeds on man principally while indoors. If the spray is having the desired effect in killing these human feeders, the *quadrimaculatus* population about sprayed premises should contain fewer human fed specimens than is the case normally.

Table 1 gives a summarization of the results of the 1946 spraying operations in keeping houses free of resting anophelines. It shows that of 6018 houses inspected up to one month after spraying, only 49 contained anophelines; thus, 99.19 per cent

of the treated houses were found to be free of these mosquitoes. At intervals of 1 to 2, 2, to 3, 3 to 4, and over 4 months after spraying, the percentages of sprayed houses free from mosquitoes decreased slightly, but even after 4 months, 98.22 per cent of the sprayed houses still were free from *quadrimaculatus.* For comparison, the percentages of *quadrimaculatus* free houses at similar intervals after spraying during the 1945 season are shown in the table. As has been stated, an application of only 100 mg. DDT per square foot was used during 1945, while the 1946 application rate was 200 mg. per square foot. The effect of the heavier dosage in increasing the duration of effectiveness of the spray is clearly indicated by the smaller percentages of *quadrimaculatus* positive houses occurring in each spray age group during 1946 as compared with 1945. In all, 21,951 sprayed houses were inspected during 1946 and approximately 99.0 per cent were free of *quadrimaculatus* mosquitoes. This may

TABLE 1

House Inspections on Residual Spray Program

Total treated houses inspected and per cent free of A. quadrimaculatus in afternoon.

MONTHS AFTER SPRAYING	1946			1945*
	Number houses inspected	Houses free of *A. quad.*		Per cent of houses free of *A. quadrimaculatus* (for comparison)
		Number	Per cent	
—1	6018	5969	99.19	98.9
1–2	6739	6673	99.02	98.3
2–3	5321	5271	99.06	95.7
3–4	2974	2935	98.69	94.7
4+	899	883	98.22	94.2
Total.	21951	21731		
Per cent.			99.00	97.2

* Bradley, G. H. and Roy F. Fritz. 1946. Entomological evaluation of DDT residual spraying for malaria control. Jour. Nat'l. Malaria Soc. **5:** 141–145.

be compared with 97.2 per cent of sprayed houses free of mosquitoes in 1945 when the lighter DDT application was made. This increased duration of effectiveness is considered highly significant since it indicates the possibility of reducing the frequency of DDT applications and thereby reducing labor costs. Thus, it appears that instead of applying DDT at intervals of 3 months, as is commonly recommended, a single spraying with a heavier dosage will provide protection from anophelines for a much longer period and at the same time will be more effective. In the more northerly malarious zones of this country, one application per season at the rate of 200 mg. per square foot may suffice and not more than two would probably be necessary anywhere in the country.

In table 2, the inspection records of both sprayed and unsprayed houses are summarized. These are arranged to show the number of houses inspected on premises having various densities of *quadrimaculatus* as indicated by the numbers of *Anopheles* occurring in favorable unsprayed resting places on the premises, and the

percentage of houses harboring these mosquitoes in each of these density groups. Included in this table are records of inspections of the 21,951 sprayed and of 1,639 unsprayed houses. The latter show the extent of normal house infestation in the presence of the various *quadrimaculatus* densities. For both sprayed and unsprayed areas, houses were considered *quadrimaculatus* positive only when mosquitoes were found in them during the afternoon. However, houses found negative in the morning have been presumed to be negative in the afternoon also. The data clearly indicate the effectiveness of the spray as applied in these large-scale operations in keeping houses free of resting mosquitoes. As might be expected, as the densities of mosquitoes about premises increase, the percentage of houses with mosquitoes also increases to a slight extent.

TABLE 2

House Inspections on Residual Spray Program

Number of house inspections in various *quadrimaculatus* density groups in sprayed and unsprayed areas and per cent of afternoon "quad.-positive" houses.

	NATURAL RESTING PLACE QUADRIMACULATUS DENSITIES						
	0–10	11–50	51–100	101–200	201–400	400+	All
Sprayed Areas							
No. houses inspected	14956	4693	1151	561	348	242	21951
No. with *A. quad*	79	63	28	17	13	20	220
Per cent	0.5	1.3	2.4	3.0	3.7	8.3	1.0
Unsprayed Areas							
No. houses inspected	1398	177	34	9	12	9	1639
No. with *A. quad*	99	77	13	7	6	6	208
Per cent	7.1	43.5	38.2	77.8	50.0	66.7	12.69

Selection of areas for residual spray operations has been based on malaria rates rather than on *quadrimaculatus* densities. However, it is of interest that of the 21,951 sprayed houses inspected, 6,995 or 32 per cent were in areas having premise densities in the groups above 10, while of the 1,639 unsprayed houses inspected only 241 or 15 per cent, were in this category. These figures indicate that in those areas in which residual spray work has been carried on (that is, in the more malarious areas) *Anopheles* densities are, in general, higher than in areas not selected for the work.

The numbers of mosquitoes which have been found in sprayed houses during afternoon inspections have been small, averaging less than 2 mosquitoes per house for the 220 afternoon positive houses. Also, it is quite likely that not all of these mosquitoes will survive as we may assume that some of them will shift from a non-lethal to a lethal surface during the evening, providing the house has been properly sprayed.

Concerning the reduction of *quadrimaculatus* positive houses from morning to afternoon, we have records of 175 sprayed houses in which a total of 318 mosquitoes

were found during the morning. Upon reinspection during the afternoon, 124 of these houses were mosquito free. Thus, a reduction in positive houses from morning to afternoon of 71 per cent occurred. In the remaining 51 houses in which mosquitoes survived until afternoon, a total of 97 mosquitoes was counted in the morning and 74 in the afternoon for a reduction of only 25 per cent. No doubt the explanation for this persistence is that these houses were not adequately sprayed since it has been found that normally fewer mosquitoes are found inside houses in the afternoon than in the morning. The meagre data we have on this point show such a reduction of about 13 per cent.

Of bloods taken during the season from 25,798 specimens of *quadrimaculatus* collected on premises where the houses had been treated with DDT residual spray, only 40 or 0.2 per cent gave precipitin reactions for human blood, while blood from 6,509 specimens collected from premises where the houses were untreated, 74 or 1.1 per cent gave human reactions. Thus, it is indicated that the proportion of the *Anopheles* population that had fed on humans, and survived, is 82.0 per cent less around sprayed than around unsprayed premises. This finding of 1.1 per cent human feedings among the unsprayed premise specimens as against 0.2 per cent among sprayed premise specimens is highly significant statistically. By chance, such a difference would be expected to occur in like samplings less than one time in 20 million. The effect of the spray work in lessening malaria transmission among humans can only be determined, of course, from epidemiological data. However, it safely may be concluded from the data presented that the chances for malaria transmission in the sprayed areas has been greatly reduced as a result of this highly effective work in the selective killing of human fed *Anopheles*.

SUMMARY

During 1946 over 750,000 houses in the southeastern United States were treated one or more times with residual DDT spray at a rate of 200 mg. DDT per square foot for malaria prevention. Inspections made of randomly selected sprayed houses indicate that up to one month after spraying 99.2 per cent were free of *quadrimaculatus*, and that this percentage had only decreased to 98.2, four months later. The greater proportion of sprayed houses found mosquito positive occurred in areas of high *quadrimaculatus* densities. By means of the precipitin test it was determined that only 0.2 per cent of the *quadrimaculatus* population around sprayed houses showed human feeding, while 1.1 per cent of those around unsprayed houses had fed on man, an indicated reduction of 82.0 per cent. It is concluded that the hazard of malaria transmission was greatly reduced as a result of the residual DDT spraying work.

RESÚMEN

Durante 1946, más de 750.000 casas en la parte sur de los Estados Undidos fueron tratadas una o más veces con tratamiento residual de D.D.T. a la dosis de 200 mg. de D.D.T. por pié cuadrado para prevensión de malaria. Se hicieron inspecciones de casas rociadas, elegidas al azar las que indicaron que hasta el fin del primer mes,

después del rociamiento, el 99.2 por ciento estaba libre de *A. quadrimaculatus* y que éste porcentaje únicamente decreció al 98.2 a los 4 meses posteriores. La mayor proporción de casas rociadas positivas para mosquitos ocurrió en las áreas de alta densidad de *quadrimaculatus.* Por medio del test de las precipitinas se determinó que únicamente 0.2 por ciento de la población del *quadrimaculatus* alrededor de las áreas en las cuales se habían rociado las casas, mostraron sangre humana, mientras que 1.1 por ciento de aquellos, alrededor de las casas no tratadas se habían alimentado en el hombre, lo que es una diferencia de 82.0 por ciento. Se concluye que el azar de las trasmisiones de la Malaria fué ampliamente reducido como resultado del trabajo de rociamiento residual de D.D.T. en las casas.

NON-PIGMENTED FORMS OF *PLASMODIUM GALLINACEUM* IN CHICK EMBRYOS: WATER COLOR PLATES

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The original description of an infection of chick embryos with *Plasmodium gallinaceum*, characterized by predominance of exo-erythrocytic forms of the parasite, and noteworthy for the appearance of non-pigmented forms in the erythrocytes, was published by the present authors in this Journal, December, 1945, Vol. IV, No. 4. Herewith are published color reproductions of water color plates done by Inez Demonet, artist of the National Institute of Health.* Technical details prevented the publication of these pictures at the time the original article appeared.

Explanation of Plates

Plate opposite page 122

Upper rectangle—Figures 1 to 10, inclusive, show progressively larger trophozoites, and a complete schizogonic series, of the non-pigmented forms of *P. gallinaceum* seen in erythrocytes of chick embryos infected by yolk sac inoculation in the manner described in the original publication. It is clear that no pigment occurs in any of these parasites, from the youngest trophozoite straight through the developmental progression to the completely segmented schizont.

Figure 11 shows a pre-segmenting schizont of the non-pigmented type in a blood cell of the erythrocytic series, from which the nucleus has disappeared. This particular host cell is evidently not a fully matured erythrocyte.

Figure 12 shows a large, compact, pre-segmenting schizont of the non-pigmented series, with no host cell visible. Forms such as these appear in embryo blood films; either such a parasite has been extruded from its host cell, or the latter has been stretched to a remnant that is too fragmentary to be seen.

Lower rectangle—Figures 1 to 10, inclusive, are the normally pigmented counterparts of the parasites shown with the same numerical designations in the upper rectangle. These parasites are seen in blood films from embryos inoculated with infected blood by the intravenous route (Haas, Feldman, and Ewing, 1945). Though pigment is usually not apparent, or difficult to discern, in the youngest trophozoites in any type of *P. gallinaceum* infection, it is evident that this feature is prominent in all the trophozoites beyond the smallest stage, and in all the schizonts, as illustrated in this series.

Figures 11 and 12 are micro- and macrogametocytes, respectively, as seen in chick embryos infected by the bites of mosquitoes (*Aedes aegypti*) containing sporozoites of *P. gallinaceum* (Haas and Ewing, 1945).

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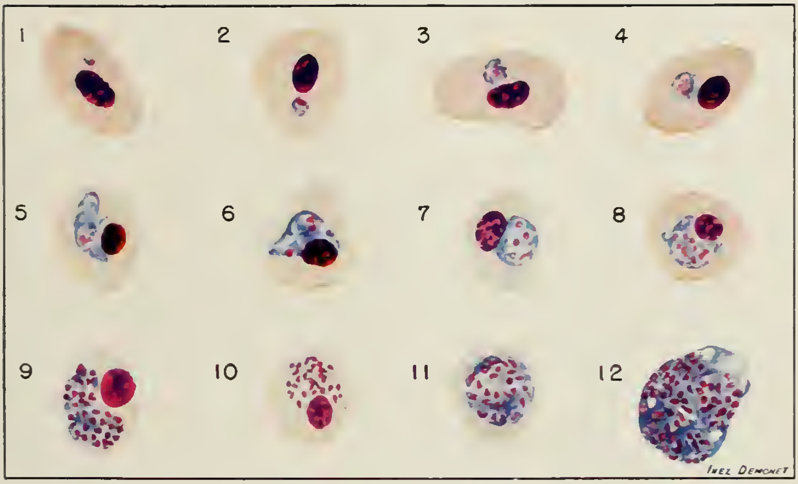
* The authors wish to express their gratitude to Miss Demonet for the painstaking accuracy with which she executed these plates. It is felt that they represent as accurate a representation of the actual appearance of these parasites under the microscope, as it is possible to attain. Another example of Miss Demonet's work with malaria parasites may be seen in Public Health Bulletin No. 180: Manual for the Microscopical Diagnosis of Malaria in Man, by Aimee Wilcox, published by the U. S. Government Printing Office, 1942.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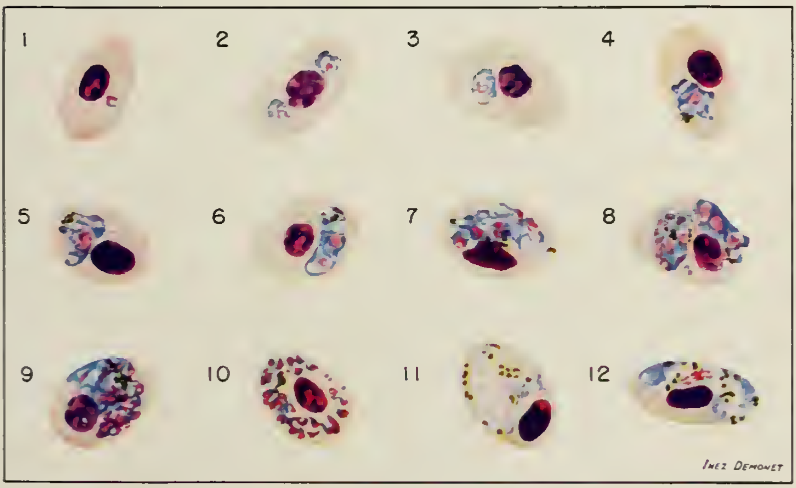
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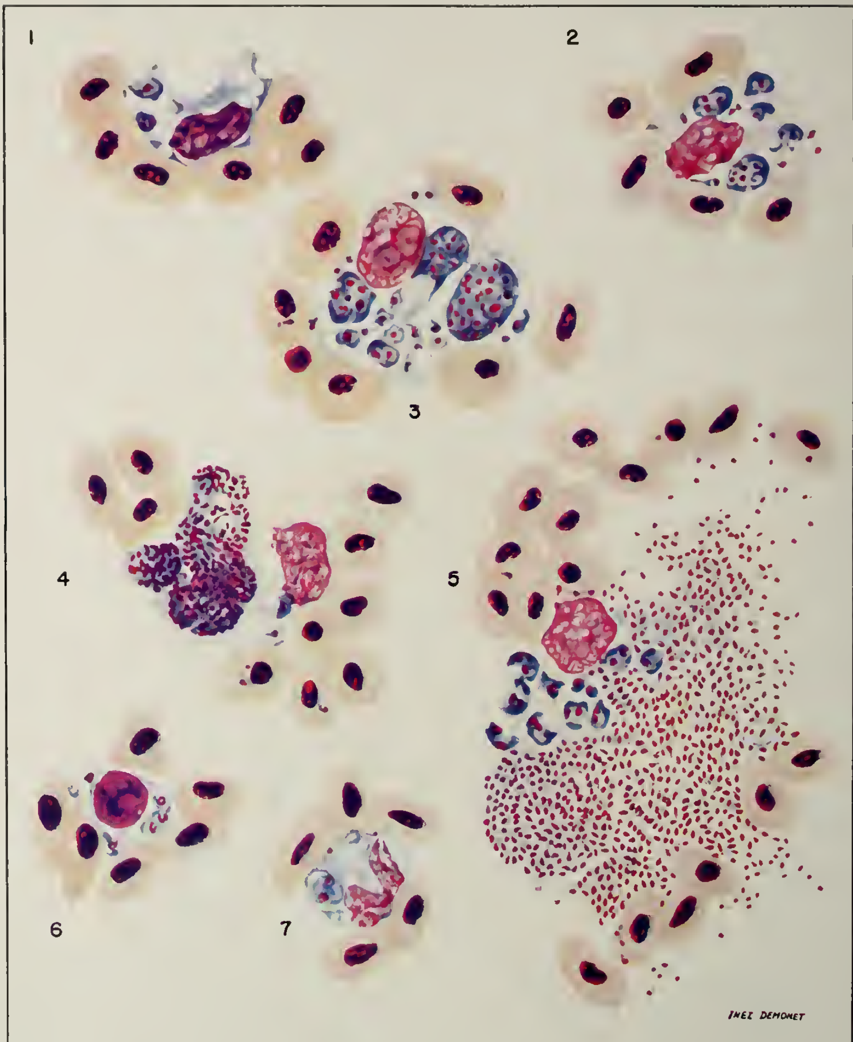
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Plate opposite page 123

Figures 1–7 show the classical type of exo-erythrocytic forms of *P. gallinaceum*, (James and Tate, 1937; 1938) as seen in blood films made from hearts of chick embryos with the exo-erythrocytic type of infection. These parasites, which include trophozoites and schizonts, may be seen to be inside cells of the lymphoid-macrophage series. Groups of erythrocytes, some containing small trophozoites, are seen around the periphery of the parasitized macrophages. Multiple parasitization is apparent in all the host cells shown.

Figure 5 shows a mature segmenting *P. gallinaceum* schizont which has burst out of the host macrophage cell, with numerous merozoites scattered about the field. Several erythrocytes in the vicinity appear to contain very young trophozoites, which bear an obvious resemblance to the merozoites of the large schizont. The host macrophage, in addition to this large segmentor, also contains several smaller pre-segmenting schizonts.

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A PRELIMINARY REPORT ON MALARIA CONTROL BY DDT RESIDUAL SPRAYING

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During World War II it became evident that there might be a marked increase of malaria in the United States as a result of introduction of the disease by returning servicemen who had contracted malaria in various parts of the world. It is estimated that over a half million of our troops acquired malaria during the war years, of which a certain unknown fraction returned as carriers, thus increasing our malaria potential by that much. This potential threatened to reverse the consistent downward trend of cases reported in this country over a period of years. It is too early to be certain whether or not the introduction of large numbers of carriers has actually increased malaria transmission in this country. However, no significant transmission has yet occurred which could be traced to importation of malaria from abroad. The downward trend of reported cases acquired within the United States is still being maintained.

To combat the possibility of transmission of malaria from returning servicemen to the civilian population, especially in the traditionally endemic malaria areas in the South, an emergency program directed primarily against adult mosquitoes was initiated by the U. S. Public Health Service during the 1945 malaria season. The activity is called the Extended Malaria Control Program and consists principally in the use of DDT as a residual spray on the interior of houses. This Extended Program is so named to distinguish it from the Malaria Control in War Areas Program which was carried on during the war to protect *military* personnel in this country from *civilian* malaria and which was the exact opposite aim of the Extended Program.

In the Extended Malaria Control Program during the calendar year 1945, approximately 400,000 houses were sprayed one or more times in 119 counties of 13 states. This work has been expanded so that approximately 750,000 houses will be sprayed one or more times in 266 counties of 13 states during the present calendar year. Figure 1 shows a map of all areas of operations in 1946.

The Extended Program is based on results of laboratory work which demonstrated the residual effect of DDT and its lethal action against adult mosquitoes. Reduction of malaria by controlling adult anophelines is not a new idea. Orenstein (1913), in Panama, reduced human malaria by employing laborers to catch adult mosquitoes daily. Russel, Knipe, and Sitapathy (1943), in India, reduced human malaria by using pyrethrum sprays to keep interiors of houses free of mosquitoes. Aitken (1946), in Italy, used DDT as a residual spray to show a remarkable effect in reducing larval and mosquito densities with a resultant reduction in parasitemia rates, size of spleens, and frequency of enlarged spleens. Trapido (1946), in Panama, used DDT as a residual spray to demonstrate a large reduction in numbers of mosquitoes both

in the village area outside of treated houses as well as in the forest area adjacent to the village. He also showed a definite reduction of positive blood smears in the sprayed village, a reduction not previously accomplished by ten years of therapeutic methods.

In the face of the present downward trend of reported malaria and in view of other factors tending to reduce malaria in the United States the effect of the Extended Program in accelerating this trend is difficult to measure. Comparison of cases reported in 1944, the last year before DDT spraying, and 1945, the first year of DDT spraying, is further complicated by the fact that many cases of military malaria are included in the figures reported for those years. Thus, it is not known how much malaria was actually acquired locally. Continuing experience is necessary before the

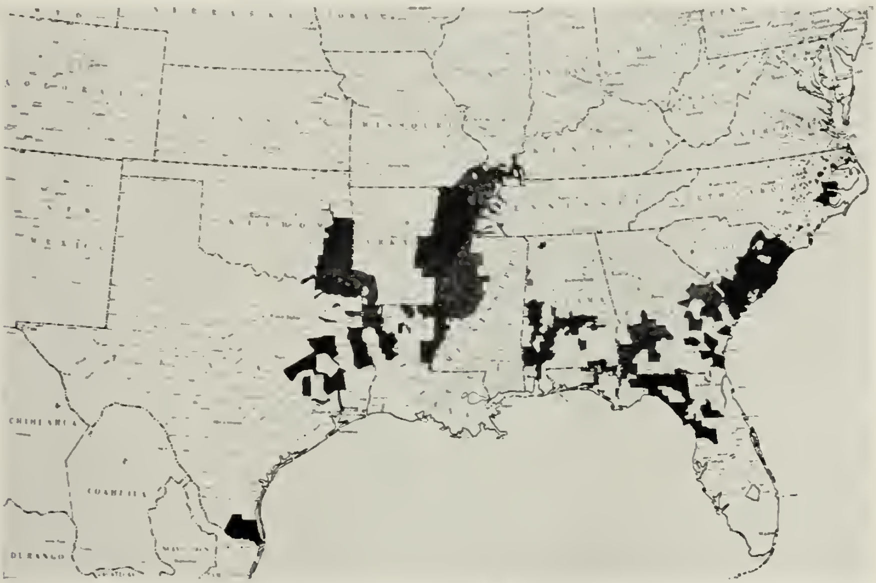

FIG. 1. COMMUNICABLE DISEASE CENTER EXTENDED MALARIA CONTROL PROGRAM AREAS—1946

effect of this type of program in decreasing malaria can be measured in terms of reported cases. However, the effect on the incidence of cases of human malaria in areas which have been sprayed with DDT has been measured in two study projects which were established in the fall of 1944 by the Office of Malaria Control in War Areas (Communicable Disease Center).

The first of these study projects was located at the Santee Cooper Reservoir in South Carolina and is a cooperative study with the South Carolina State Board of Health. Figure 2 shows the location of this project. This region was chosen because it represented the only known high endemic malaria area in the United States. Nearly 20 per cent of the population showed positive blood films in October 1944. A portion of the area adjacent to the reservoir was divided into approximately equal parts, one of which was sprayed, the other left unsprayed. Each part has a population of approximately 1500 people of whom 90 per cent are Negroes. Most of the

people work on farms and live in homes which are not mosquito proofed. A preponderance of the malaria in the area is caused by *Plasmodium falciparum*. *Anopheles quadrimaculatus* is the important mosquito vector in this area.

Houses were sprayed with DDT during the last two weeks of April 1945, and again during the last two weeks of July 1945.

The criterion used to measure the amount of malaria present is the thick blood film. Pre-spraying blood surveys were conducted in October 1944 and in April 1945. Post-spraying surveys were carried out at monthly intervals beginning in June 1945, and are still continuing. Figure 3 and table 1 summarize the results of the blood

Fig. 2. Location of Manning, South Carolina DDT Residual Spray Project

surveys. Comparison of the two areas showed that the malaria prevalence in the unsprayed area rose during the transmission season while remaining at a stationary level in the sprayed area. There was a statistically significant difference between the prevalence in the two areas. In spite of the low malaria prevalence during the 1945 season, the validity of this difference is corroborated by comparison of infection rates in the age group under ten where the difference is greater than that of the age group over ten (Reider et al., unpub.).

The second project established for the purpose of determining what effect DDT has on human malaria was located in Puerto Rico. This is a joint project of the School of Tropical Medicine, Insular Health Department, and the U. S. Public Health Service District Number 6. Epidemiological, entomological, and engineering assistance in starting the study was supplied by the office of Malaria Control in War Areas. This study is of especial interest because of the fact that the important malaria vector in Puerto Rico is *Anopheles albimanus*, a "wild" mosquito whose habits differ from

those of the vector found in the United States as it seldom remains within houses except for a few hours during the night.

Two villages, Humacao Playa and Loiza Aldea, situated in the northeast corner of Puerto Rico, were selected for the study. Figure 4 shows the location of these

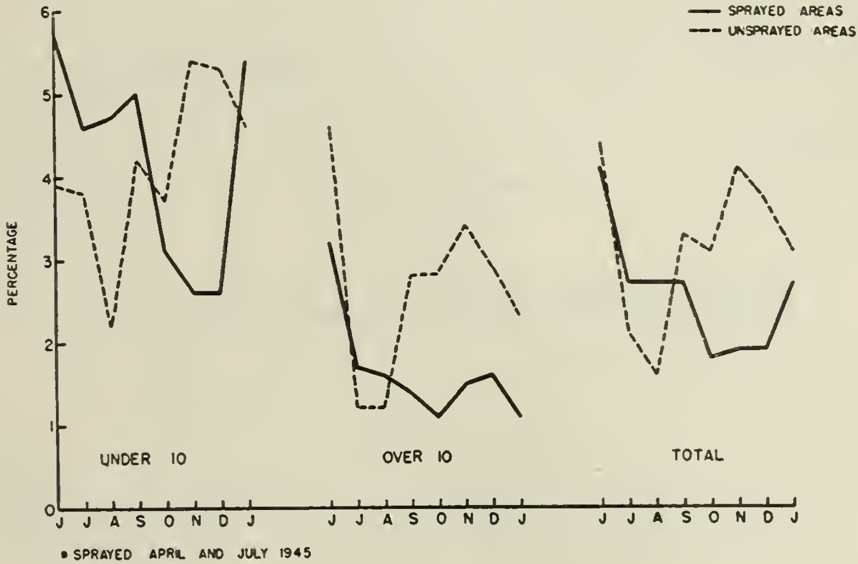

Fig. 3. Percentage Positive Blood Slides by Age Groups in Sprayed* and Unsprayed Areas—South Carolina, June 1945 to January 1946

TABLE 1

*Per cent positive blood slides by age groups, South Carolina, June 1945–January 1946**

MONTH OF SURVEY	SPRAYED AREA									UNSPRAYED AREA								
	Under 10			Over 10			Total			Under 10			Over 10			Total		
	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%
June	580	33	5.7	1110	36	3.2	1690	69	4.1	462	18	3.9	883	41	4.6	1345	59	4.4
July	592	27	4.6	1110	19	1.7	1780	46	2.7	452	17	3.8	835	10	1.2	1287	27	2.1
August	593	28	4.7	1079	17	1.6	1672	45	2.7	460	10	2.2	808	10	1.2	1268	20	1.6
September	564	28	5.0	1055	15	1.4	1619	43	2.7	426	18	4.2	752	21	2.8	1178	39	3.3
October	573	18	3.1	1064	12	1.1	1637	30	1.8	434	16	3.7	798	22	2.8	1232	38	3.1
November	570	15	2.6	1002	15	1.5	1572	30	1.9	447	24	5.4	815	28	3.4	1262	52	4.1
December	576	15	2.6	1027	16	1.6	1603	31	1.9	435	23	5.3	795	23	2.9	1230	46	3.7
January	589	32	5.4	1001	11	1.1	1590	43	2.7	417	19	4.6	767	18	2.3	1184	37	3.1

* Courtesy of Surgeon (R) R. F. Reider, Medical Officer In Charge, Malaria Investigations Project.

villages on the Island of Puerto Rico. Humacao Playa was chosen as the sprayed village and Loiza Aldea was left unsprayed. These villages are similar enough in all of the factors involved in malaria transmission to be acceptable as comparable areas. Spraying was accomplished in November 1944, July 1945, and November 1945 and consisted of premise spraying of houses and outbuildings.

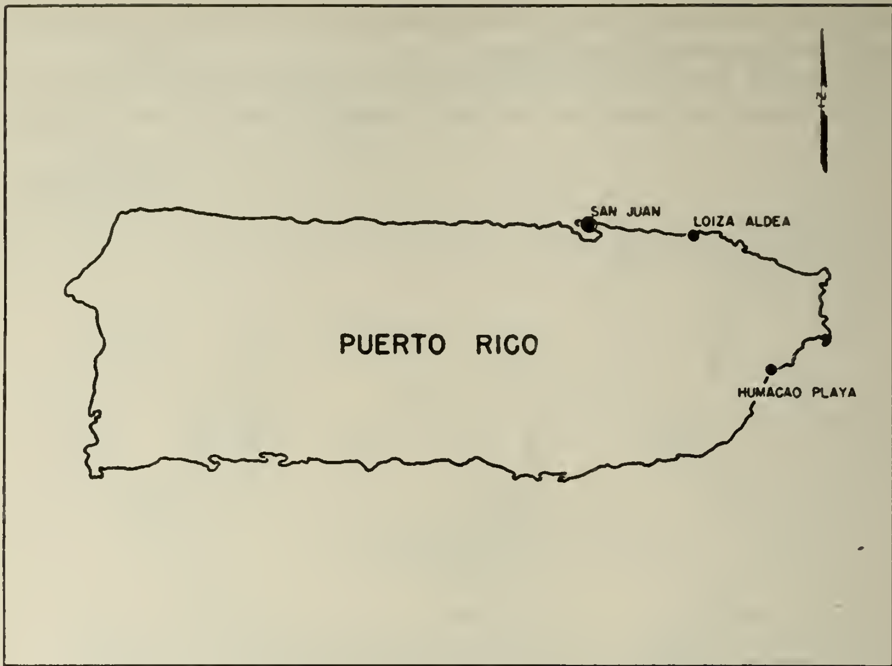

Fig. 4. Location of Puerto Rico DDT Residual Spray Project

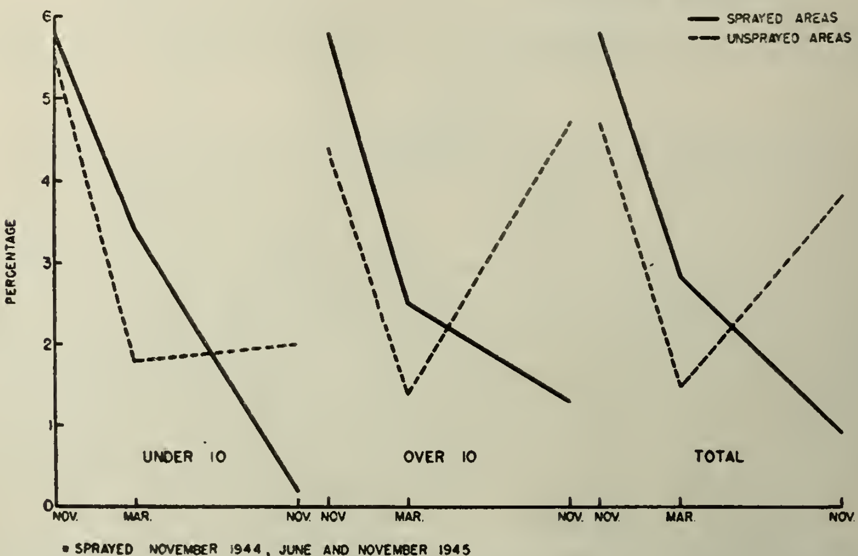

Fig. 5. Percentage Positive Blood Slides by Age Groups in Sprayed* and Unsprayed Areas—Puerto Rico, November 1944 to November 1945

Animal bait trap and light trap collections which were made throughout the year showed that *albimanus* was present in sufficient numbers during the study period to

transmit malaria. From one to several hundred *albimanus* were collected from all traps during each night of operation.

Three blood film surveys were made in the late fall of 1944, in early spring of 1945 and the late fall of 1945. The results of these surveys are shown in summary on figure 5 and table 2. It will be noted that both villages show a decrease in positive smears in the second survey which represents the normal seasonal decline in the incidence of malaria transmission. On the third survey, however, a very significant difference in positive blood smears is seen in which the sprayed village showed only 0.9 per cent positive smears while the unsprayed village showed 3.8 per cent positive smears. The 0–9 year age group in the sprayed village shows the greatest decrease of all in the third survey. Since positivity in younger age groups is often used as an

TABLE 2

*Per cent positive blood slides by age groups, Puerto Rico, November 1944 to November 1945**

SURVEY	HUMACAO PLAYA (SPRAYED)									LOIZA ALDEA (UNSPREAYD)								
	Under 10			Over 10			Total			Under 10			Over 10			Total		
	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%	Slides	Pos.	%
Nov. '44	483	28	5.8	977	57	5.8	1460	85	5.8	380	21	5.5	891	39	4.4	1271	60	4.7
Mar. '45	440	15	3.4	762	19	2.5	1202	34	2.8	276	5	1.8	576	8	1.4	852	13	1.5
Nov. '45	406	1	0.2	687	9	1.3	1093	10	0.9	292	6	2.0	576	27	4.7	868	33	3.8

* Courtesy of Sanitary Engineer (R) Porter Stephens, C.D.C. District Representative, San Juan, Puerto Rico.

index of transmission, it is considered to be especially significant that so few positives were recorded in this group in comparison with the number of positives in the same age group of the unsprayed village (Stephens and Pratt, unpub.).

SUMMARY

1. The Extended Malaria Control Program is designed to reduce malaria transmission by using the residual effect of DDT against adult mosquitoes.

2. The actual effect on human malaria of the Extended Malaria Program in the United States is impossible to measure at this time. Further observation is required before conclusions can be drawn as to the overall effect on human malaria in the program areas in this country.

3. Results so far obtained from two study projects indicate that spraying of homes with DDT decreases the incidence of human malaria.

RESÚMEN

1º—El programa extensivo de control de Malaria se designa para reducir la transmisión de la Malaria, usando los efectos residuales del D.D.T. contra los mosquitos adultos.

2º—El efecto actual sobre Malaria humana del programa malárico extensivo en los Estados Unidos es imposiblede medir en ésta época. Observaciones posteriores se requieren antes de que se puedan sacar conclusiones sobre el efecto completo sobre la malaria humana en las áreas en que se ha hecho el trabajo en este país.

3°—Los resultados hasta ahora obtenidos de dos proyectos de estudio, indican que el rociamiento de las casas con D.D.T. hace bajar la incidencia de la Malaria humana.

ACKNOWLEDGMENT

Acknowledgement is due to Surgeon (R) R. F. Reider, Medical Officer in Charge, Malaria Investigations Project, Manning, South Carolina, for providing data used in figure 3 and table 1.

Similar acknolwedgement is due to Sanitary Engineer (R) Porter A. Stephens, U. S. Public Health Service District Number 6, San Juan, Puerto Rico, for providing data used in figure 5 and table 2.

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AN APPRAISAL OF MALARIA REDUCTION IN ALBANIA, 1929–1938*

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Four years after the inauguration of antilarval measures for the control of malaria in Sardinia and Italy similar projects were initiated in Albania on the invitation of King Zog to the International Health Division of The Rockefeller Foundation. The Malaria Bureau was organized in 1929 as a part of the Health Department under the direction of Dr. Anton Ashta. The object of the present paper is to describe the malaria situation in Albania and to appraise statistically the results of the projects. The biometers and the statistical techniques employed were defined in appraising the Sardinian projects (Putnam and Hackett, 1946) and will not be discussed again here.

MALARIA IN THE BALKANS

During the period covered by the Albanian projects similar studies were in progress in Bulgaria and Greece; thus the malaria problem in the Balkans during the 1930's was investigated, and it has been described by one of us (Hackett, 1937). The physical conditions under which men live and work in these countries are harsh and primitive and the resources are correspondingly meager, so that antimalaria work must be carried out with the means at hand and in large part with funds locally available.

The problems of the control of anopheline breeding which beset the malarious villages of the Balkans differ in kind and magnitude and must be solved, if at all, by ingenuity and imagination without the aid of modern engineering devices. Little reliance can be placed on the ability of the local population to maintain complicated larvicide services year after year, consequently a permanent solution should be sought for each malaria problem, even if the initial expenditure may seem out of proportion to the local means available and likely to limit rapid extension of the program.

ALBANIAN VECTORS OF MALARIA

The anopheline problem of Albania is similar to that prevailing throughout the Balkans. Geographically it may be described as follows: *Anopheles sacharovi* was found breeding prolifically in the salty lagoons of the coastal plains and was known to be an important vector of malaria. *Anopheles messeae* appeared to be a highland species while *Anopheles maculipennis* (typicus) and *Anopheles melanoon subalpinus* were found in every region in which collections were made. These may not be important vectors of malaria, but in areas where *Anopheles superpictus* or *Anopheles sacharovi* also abound they may play a part in the transmission of the

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation and the Seksioni Antimalarik in Albania.

disease. *Anopheles superpictus* was found breeding extensively in the gravel beds of the torrential streams during the dry summer season. Bates (1937) has described the factors influencing the seasonal prevalence of anophelines in the neighborhood of Tirana, the capital of Albania, where studies were first initiated. He noted that *A. maculipennis* (typicus) was responsible for the midsummer peak in density while subalpinus maintained a constant level throughout the season. In this paper these two races will not be differentiated but will be referred to as "maculipennis."

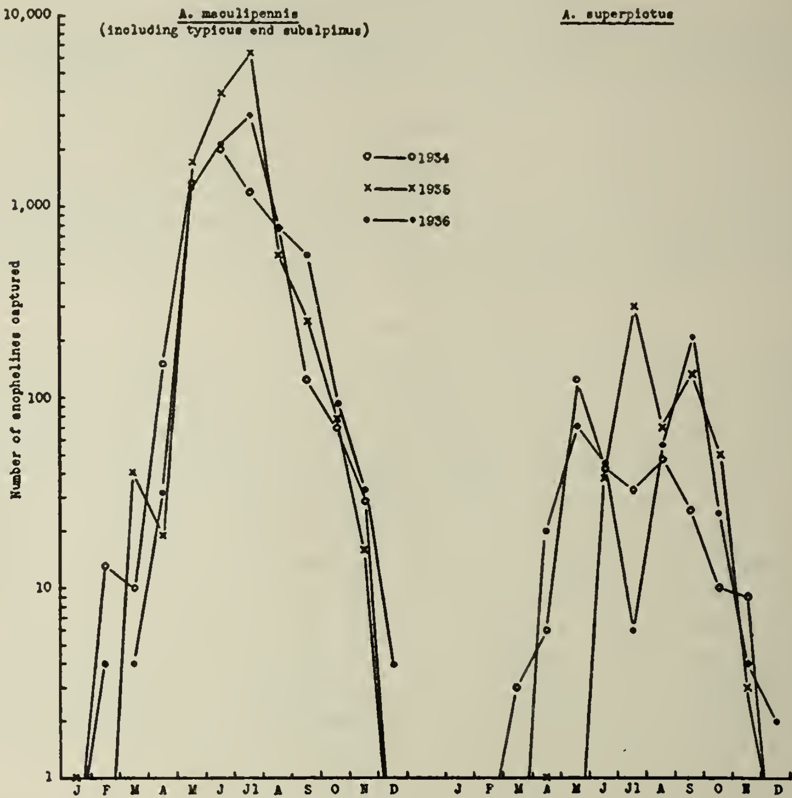

FIG. 1. CAPTURES OF ANOPHELINES BY MONTH AND YEAR AT TWO STATIONS OUTSIDE THE PROTECTED AREA OF TIRANA

The seasonal waves of maculipennis and superpictus are plotted in figure 1 on a semilogarithmic scale so that relative differences may be elicited. These data are based on captures in two stables outside the protected area of Tirana during the years 1934–1936. Figure 2 presents the corresponding picture of sacharovi prevalance based on captures at the periphery of the protected area in Valona. The three species differ radically as to their seasonal distribution. Maculipennis appears early and rises to a high peak in June and July with captures continuing throughout the year. The year 1936 was rainy and was considered a bad one in so far as malaria prevalence was concerned; but the actual level of the maculipennis peak for the year did not

exceed that of 1935. In fact the area under the curves was similar in the three seasons shown.

The seasonal contour of the superpictus waves, on the other hand, was quite irregular. At least two peaks are distinguishable in each year. Captures of superpictus were made throughout the year, but the peak density attained was never more than one-third that of maculipennis in this area. Captures of sacharovi, on the other

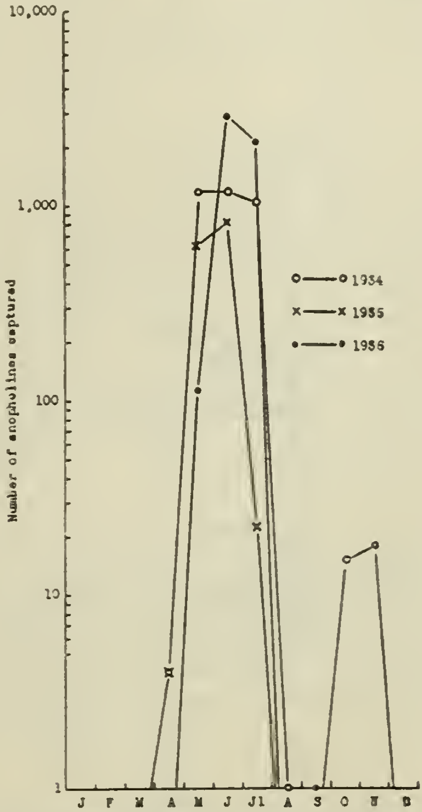

Fig. 2. A. sacharovi Captures by Month and Year at Four Stations on the Periphery of the Protected Area of Valona

hand, were limited in 1935 and 1936 to virtually three months, May, June, and July. In 1934 a secondary rise of minor importance occurred in October and November. The shortness of the season for sacharovi is due to the limited range of salinity which it can tolerate. During the dry season the salinity rises until it reaches a level at which sacharovi can no longer breed.

ANTILARVAL PROJECTS

The projects to be considered here are those at Tirana, which is the capital of Albania, its port, Durazzo, two inland towns of Elbasan and Berat, and a second coastal town of Valona. Antilarval measures were inaugurated in all these areas over vary-

ing periods of time. The town of Kavaja, situated not far from Durazzo, was used as a comparison area in which surveys for malaria prevalence were made annually during the entire period of the study, 1929–1938. Another town, Scutari, in which a successful campaign was carried out is not included in this review. The accompanying map of Albania shows the location of these towns.

MAP OF ALBANIA

Superpictus, possibly assisted by maculipennis, was the malaria vector of Tirana, Elbasan, and Berat, while sacharovi was primarily responsible for malaria in Durazzo and Valona. Antilarval measures in each area had to be adapted to the breeding preferences of the vector. No captures were made in the vicinity of Kavaja, but it is believed that superpictus rather than sacharovi was the dominant vector there.

Initial surveys were made in Tirana and Durazzo in 1929. The anopheline problem revealed and the steps eventually taken to solve it have been described (Hackett, 1937). Paris green was distributed routinely until permanent measures of larva control became effective. At Tirana swamps were drained, and the river bed within the protected area was eventually drained by turning all water above the

town into a well-regulated irrigation system throughout the breeding season. At Durazzo the salinity of the lagoon was raised by admitting sea water at high tide. A canal at the southern end kept the level of the lagoon and its salinity constant.

At Elbasan Paris green was applied liberally to the three river beds while efforts were made to improve the irrigation system and to restrict rice culture in the neighborhood of the town. Permanent control was not achieved however. River breeding was the chief source of malaria at Berat, where Paris green was the only antilarval measure applied. At Valona a beginning was made toward filling and draining the swamps and lagoons; but progress was slow, and the work had not been completed at the end of the period.

MATERIAL AND METHODS OF ANALYSIS

Annual surveys in February or March of school children from 5 to 12 years of age during the period of observation furnish data for the biometers needed for appraising the effect of the antilarval measures upon the malaria endemic. These comprise parasite and spleen rates, the average spleen and the average enlarged spleen, and for completeness the average spleen of children with parasites for comparison with that for children with no parasites at the time of the examination. Since the surveys were made in the late winter the amount of malaria carried over from the previous season is indicated by these biometers. In Tirana, Durazzo, and Kavaja surveys were made annually from 1929–1938. Data for Elbasan and Berat have been combined and pertain to the years 1932–1938. In Valona the period of observation was from 1933–1938.

Methods employed in the statistical analysis of the malaria indexes pertaining to the Sardinian projects (Putnam and Hackett, 1946) are used in this paper. They comprise: the graphic presentation of annual rates and indexes on a semilogarithmic scale so that relative differences are revealed; the fitting of straight lines by least squares to the logarithms of the biometers so that rates of decrease (b constants) as well as levels (a constants) may be compared; variance analysis for comparing the means of subgroups with that of the total.

RESULTS

Time changes and differences in level of the malaria biometers.—At the beginning of observation the spleen rates ranged from 42 per cent in Tirana to 69 per cent in Valona. During the period, these rates decreased significantly in all areas except Kavaja. In Tirana there was a drop of 30 per cent in the rate, while in other protected areas one of 20 per cent occurred. The average annual rates of decrease, shown in table 1, varied from 1.62 per cent in Kavaja, the comparison area, to 12.9 per cent in Tirana. The percentage of children with enlarged spleens, which is the least sensitive of the biometers, decreased significantly in the protected areas during the period of observation.

Parasite rates based on winter surveys of school children were never as high as spleen rates in the Mediterranean countries where studies were made. In Albania the parasite rates on the average were about one-fifth the spleen rates. Only once,

in 1933, did the parasite rate in Elbasan and Berat attain a level of one-third the spleen rate. Parasite rates fluctuated sharply from year to year as shown in figures 3 and 4; and although the annual percentage decreases, with the exception of that for Valona, were somewhat greater than those of the spleen rates, the differences were never significant. Again the rate in the comparison town of Kavaja did not decrease significantly.

The fluctuations of the average spleen parallel those of the spleen rate but are more pronounced, and the averages decreased more rapidly than did the spleen rates

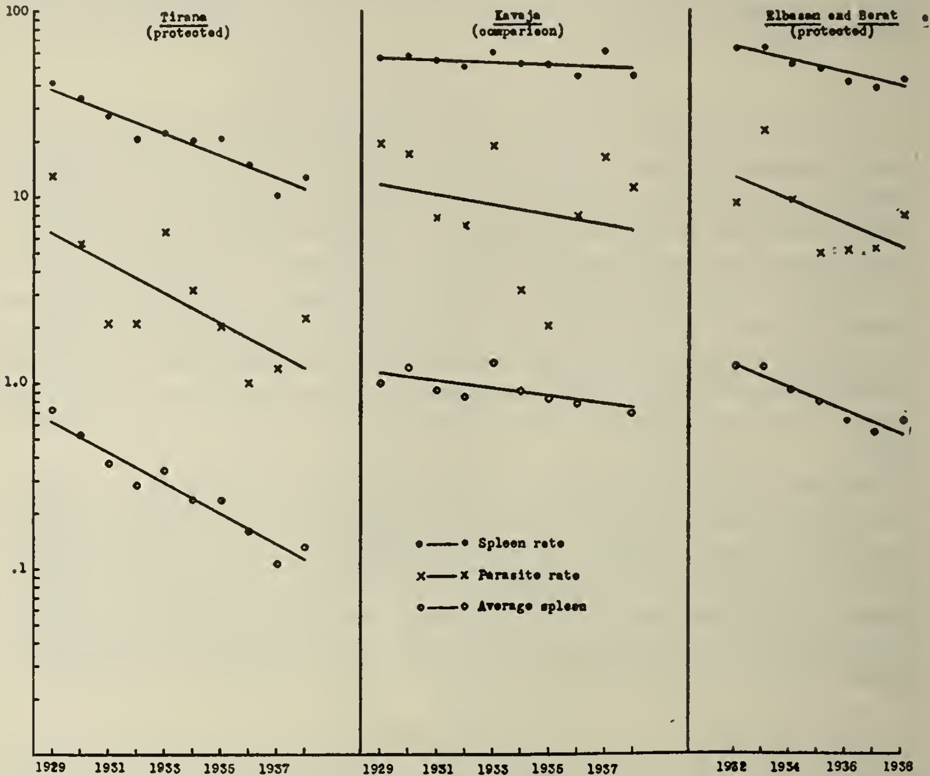

FIG. 3. TIME CHANGES IN MALARIA BIOMETERS IN AREAS WHERE A. SUPERPICTUS WAS THE CHIEF VECTOR

The average annual rates of decrease ranged from 4.5 per cent for Kavaja—which is significant—to 17.4 per cent for Tirana. It is apparent, therefore, that even in the comparison town the amount of malaria in children declined somewhat during the period of observation.

Figures 3 and 4 indicate that the levels of the biometers differ from area to area. The indexes for Tirana and Durazzo, where permanent preventive work was done, lie below those of the other areas. While the change with time was least in Kavaja, the comparison area, the levels of the indexes for Elbasan, Berat, and Valona as indicated by the *a* constants were actually higher.

A comparison is made in figure 5 between the level and the change with time of the average spleens of children with parasites and of those with no parasites at the time of the examination. It should be noted that the group with parasites diminished as children passed from parasite positive to negative groups, while the group with no parasites was correspondingly augmented. This shift in basic population explains partly the extreme fluctuation of the averages for children with parasites and the stability of the averages for those without them.

The fluctuations of the average spleen for children with no parasites for Kavaja

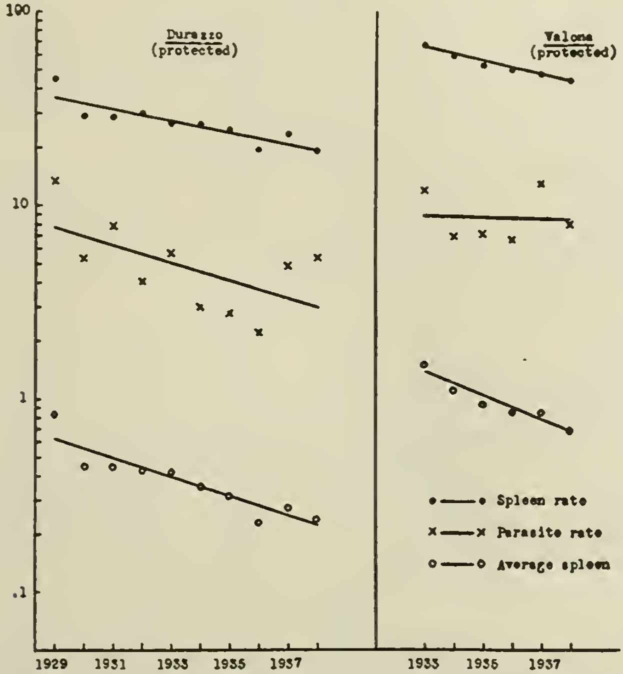

FIG. 4. TIME CHANGES IN MALARIA BIOMETERS IN AREAS WHERE A. SACHAROVI WAS THE CHIEF VECTOR

parallel those of the corresponding averages for Tirana, an observation which suggests that Kavaja was probably a superpictus area. Differences between slope and level of the biometers for the two towns are conspicuous, however. Figure 5 also enables one to compare the trend of the biometers for Tirana with those for Elbasan and Berat for the years 1932–1938. The annual rates of decrease for the average spleens of children with no parasites were similar for these areas (Tirana 15.6 per cent, Elbasan and Berat 13.1 per cent, table 1) but the average level (*a* constant) for Tirana was one-third that of Elbasan and Berat (0.37 versus 1.33). Less difference in level is apparent for children with parasites, and the averages for Tirana fluctuate sharply as the number of children with parasites included in the index is diminished.

A similar comparison may be made of the level and time trend of the two series of average spleens for Durazzo and Valona for the years 1933–1938. Similar fluctua-

TABLE 1

Constants of Regression Equations Fitted to the Logarithms of the Malaria Biometers for Varying Periods of Observation

FROM YEAR INDICATED THROUGH 1938	AREA	REGRESSION CONSTANTS			
		a		b	
		Logarithm	Per cent or Index	Logarithm	Annual rate of decrease Per cent
		Spleen rate			
1929	Tirana	1.64 ± 0.020	43.8	−0.0598 ± 0.0069	12.9
1929	Kavaja	1.76 ± 0.015	57.2	−0.0071 ± 0.0051	1.62
1929	Durazzo	1.60 ± 0.017	39.4	−0.0310 ± 0.0058	6.88
		Parasite rate			
1929	Tirana	0.903 ± 0.025	8.00	−0.0821 ± 0.0276	17.2
1929	Kavaja	1.105 ± 0.108	12.5	−0.0276 ± 0.0378	6.15
1929	Durazzo	0.928 ± 0.062	8.47	−0.0450 ± 0.0216	9.84
		Average spleen, all children			
1929	Tirana	−0.119 ± 0.023	0.761	−0.0832 ± 0.0081	17.4
1929	Kavaja	0.068 ± 0.022	1.17	−0.0205 ± 0.0085	4.50
1929	Durazzo	−0.153 ± 0.021	0.703	−0.0501 ± 0.0073	10.9
		Average spleen, children with parasites			
1929	Tirana	0.263 ± 0.063	1.83	−0.0793 ± 0.0804	16.7
1929	Kavaja	0.297 ± 0.033	1.98	−0.0279 ± 0.0119	6.23
1929	Durazzo	0.123 ± 0.087	1.33	−0.0399 ± 0.0303	8.78
		Average spleen, children with no parasites			
1929	Tirana	−0.174 ± 0.022	0.669	−0.0785 ± 0.0077	16.5
1929	Kavaja	1.01 ± 0.018	1.03	−0.0158 ± 0.0065	3.57
1929	Durazzo	−0.181 ± 0.022	0.659	−0.0507 ± 0.0077	11.0
		Spleen rate			
1932	Elbasan and Berat	1.83 ± 0.012	67.9	−0.0364 ± 0.0059	8.03
1933	Valona	1.85 ± 0.005	71.5	−0.0343 ± 0.0031	7.60
		Parasite rate			
1932	Elbasan and Berat	1.15 ± 0.080	14.0	−0.0620 ± 0.0403	13.3
1933	Valona	0.954 ± 0.059	9.00	−0.0047 ± 0.0347	1.07
		Average spleen, all children			
1932	Elbasan and Berat	0.160 ± 0.019	1.45	−0.0629 ± 0.0094	13.5
1933	Valona	0.197 ± 0.016	1.57	−0.0611 ± 0.0094	13.1

Table 1.—*Continued*

ROM YEAR INDICATED THROUGH 1938	AREA	REGRESSION CONSTANTS			
		a		b	
		Logarithm	Per cent or Index	Logarithm	Annual rate of decrease Per cent
		Average spleen, children with parasites			
1932	Elbasan and Berat	0.327 ± 0.013	2.13	−0.0457 ± 0.0063	9.98
1932	Tirana	0.136 ± 0.031	1.37	−0.1012 ± 0.0495	20.8
1933	Valona	0.310 ± 0.041	2.04	−0.0352 ± 0.0241	7.78
1933	Durazzo	0.220 ± 0.135	1.66	−0.1025 ± 0.0792	21.0
		Average spleen, children with no parasites			
1932	Elbasan and Berat	0.123 ± 0.017	1.33	−0.0608 ± 0.0084	13.1
1932	Tirana	−0.430 ± 0.032	0.372	−0.0739 ± 0.0161	15.6
1933	Valona	0.185 ± 0.009	1.53	−0.0662 ± 0.0054	14.1
1933	Durazzo	−0.409 ± 0.014	0.390	−0.0431 ± 0.0080	9.45

tions in the biometers of these sacharovi areas may be observed. During this period the annual rate of decrease in the averages for children with no parasites was less rapid in Durazzo than it was in Valona, but the level (*a* constant) for the Durazzo average was only about one-fourth that for Valona.

It is apparent that the general level of the biometers in the areas where antimalaria work began in 1929 was lower than it was in the areas where preventive measures were inaugurated at a later date or where none were attempted during the period of observation. However, even in the comparison area, Kavaja, significant decreases occurred in the measures of average spleen.

Malaria prevalence and anophelism.—Monthly data on acute attacks of malaria were not routinely available in Albania as they were in Sardinia, so comparisons between vector prevalence and malaria must be made on an annual basis. For this purpose captures at the periphery of the protected zones were used for comparison with the parasite rates for the areas studied. The assumption is that a rise or fall in anopheline prevalence on the periphery will be reflected in the parasite rates of children within the town even when no captures are made within the zone of control. Prior to 1932 anopheline captures were reported for protected and for unprotected zones only. Also no differentiation into species was made. This is unfortunate, since 1932 was considered a peak superpictus year and it would be of interest to be able to show this rise, which is reflected in the parasite rates for 1933. Figure 6 contains the annual captures per station for total anophelines and by species for Tirana separately and for Berat and Elbasan combined, although captures for Berat are limited to the years 1934–1937. Since the parasite rates reflect the malaria incurred during the previous year they have been shifted one space to the left in plotting, for comparison with the captures.

The biometers have been plotted on a semilogarithmic scale in all the figures in this paper so that similar relative changes with time might be noted. Inasmuch as the reproduction of anophelines is a multiplicative process, this method of presenting the data is the logical one to use; but it has the disadvantage of exaggerating fluctuations at low values of the biometers. An instance of this will be noted presently.

Total captures in Tirana dropped precipitously during the first three years of ob-

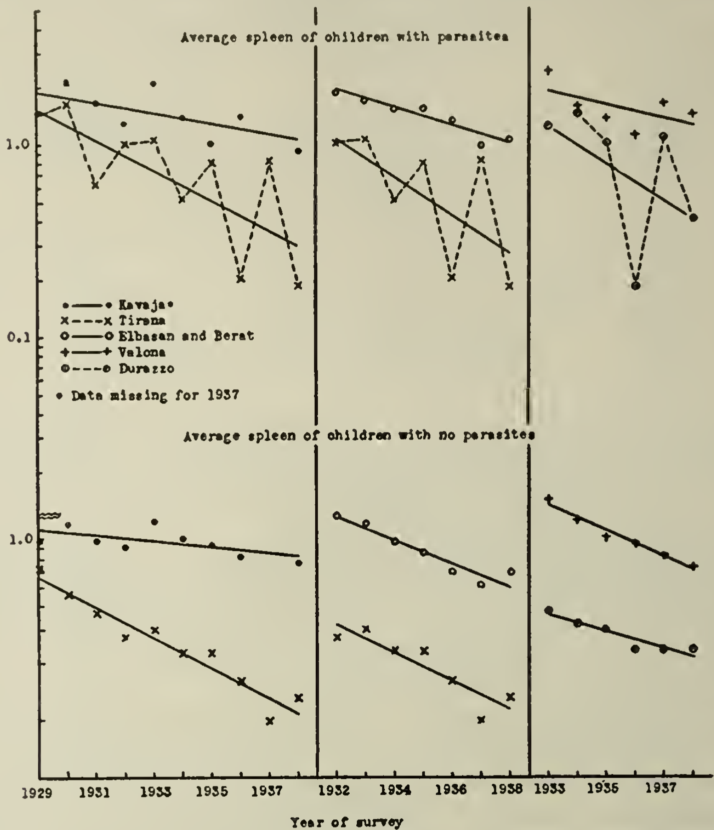

Fig. 5. A Comparison by Area of Time Changes in Average Spleens of Children with and without Parasites at the Time of the Examination

servation, but from 1931–1937 there was little change in level. The year 1932 was very dry, so maculipennis captures were low; but they rose subsequently and remained constant during the remainder of the period. This was not true of superpictus, however. Its high point was attained in 1932, and the trend was sharply downward thereafter. The rises in 1935 and 1937 were more apparent than real since they were derived from small numbers (38 and 10 anophelines being the total number captured in the respective years). The drop in the parasite rates corresponding to the capture years, 1932–1937, was not as rapid as that of superpictus,

but one cannot evaluate the effect on this rate of maculipennis or of the residual malaria carried over from year to year.

High density of both maculipennis and superpictus occurred in the years 1932 and 1936 in Berat and Elbasan, with the overall decline of superpictus being some-

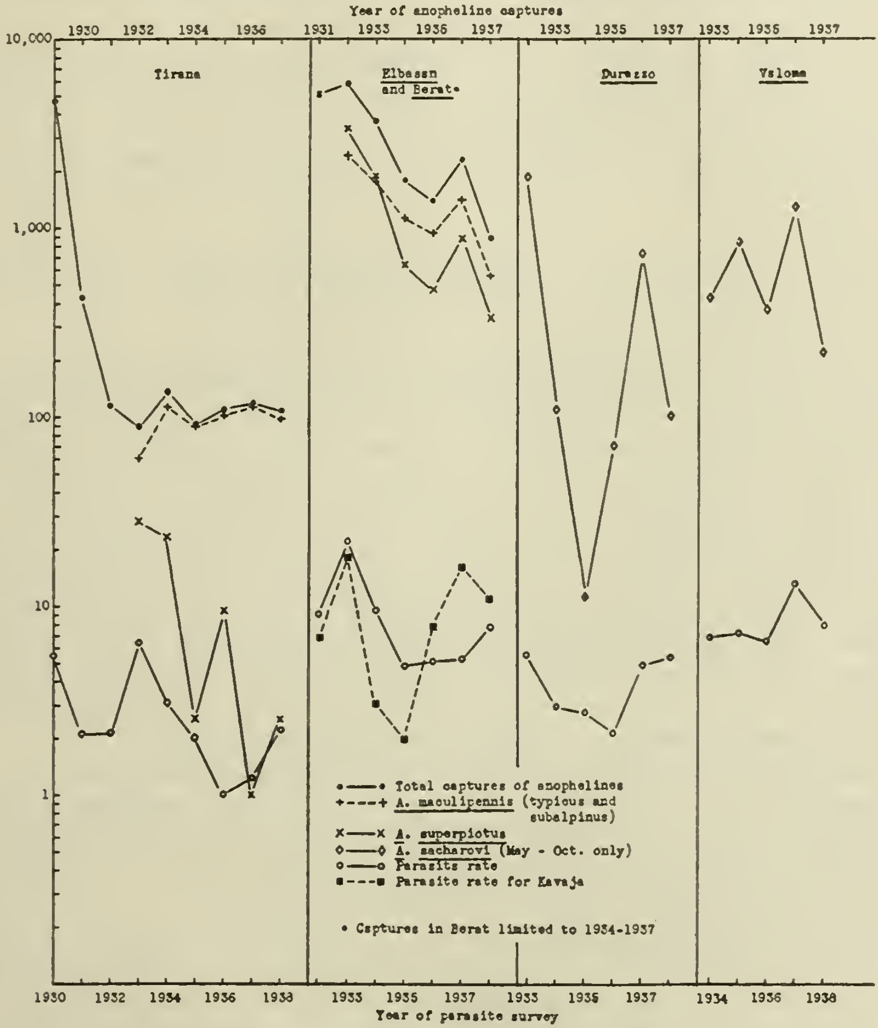

FIG. 6. YEARLY CAPTURES OF ANOPHELINES PER STATION AT THE PERIPHERY OF THE PROTECTED AREA FOR COMPARISON WITH PARASITE RATES

what greater than that of maculipennis. The parasite rate in these two towns indicated a rise in malaria in 1932 but not in 1936. Kavaja, the comparison town, although located nowhere near either Elbasan or Berat exhibits fluctuations in the parasite rate similar to those given by the captures in Elbasan and Berat for the cor-

responding period but without the downward trend. This is of interest as again suggesting that superpictus rather than sacharovi was the chief vector there.

Anophelism in Berat and Elbasan was obviously more intense throughout the period than it was in Tirana, which may account for the higher level of the malaria biometers in these communities.

A comparison is also made in figure 6 of sacharovi captures in Durazzo and Valona and the parasite rates there. The parallelism between fluctuations of the two curves is more sharply defined in these towns. The rise in anopheline prevalence at the periphery of Durazzo in 1936 occurred because the outlet to the lagoon had become clogged so that salinity was reduced to the point where sacharovi could breed again. Captures at the center of the protected area were virtually zero from 1934–1937 in this town.

This was never the case at Valona and the parallelism between captures at the periphery and the parasite rate is marked. No significant decrease in either of these indexes is apparent for this town.

Evidence of a close relationship between vector prevalence and parasite rate is limited to the case of Valona. The rapid decrease in the parasite rate in Tirana suggests that superpictus was the dominant vector. In Elbasan and Berat, however, captures of maculipennis and superpictus pursued a similar course so that a primary association of the parasite rate with superpictus density can merely be inferred.

Prevalence and relative frequency of each plasmodium in superpictus areas.—In the analysis of the Sardinian data the significance of specific plasmodial infections, as indicated by the examination of thick blood smears obtained during the winter surveys, was given careful consideration. Here, to obtain a group large enough to furnish stable rates for each plasmodium, data for the protected towns of Tirana, Elbasan, and Berat were combined with those of Kavaja on the assumption that superpictus was probably the vector in the latter town also. Prevalence rates for the three plasmodia are charted in figure 7 for the years 1932–1938.

Although the frequency of *Plasmodium falciparum* was greater than that of *Plasmodium vivax* in the survey of 1932, the latter attained the same level of more than eight per cent in 1933 and occupied a similarly important position thereafter. Infections with *Plasmodium malariae* were observed less frequently throughout the period but their frequency increased during the last three years.

The relative frequency of specific infections is given in table 2. Falciparum infections declined from 61 per cent of the total malaria infections in 1932 to a low of 30 per cent in 1934, while the average for the period was 43 per cent of the total. In 1932 vivax infections constituted only 21 per cent of the total but increased to 56 per cent in 1934 and exhibited an average for the period of 44 per cent. A low point of three per cent was recorded for malariae infections in 1933; the proportion rose to 26 per cent in 1936, while the average for the period was 13 per cent.

Similar results were obtained in the analysis of the Sardinian data, and their significance is not clear. It seems probable that multiple infections frequently occur among children in these highly malarious districts and that the diagnosis at a given survey depends upon the species circulating in the peripheral blood at the time of

the examination. In these Albanian towns the annual fluctuations in the frequency of falciparum and vivax infections were similar, while infections with malariae increased both in absolute and in relative frequency as the period advanced.

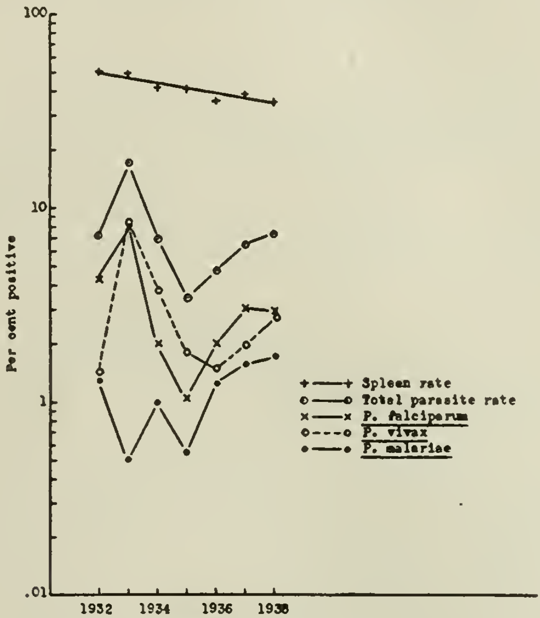

FIG. 7. RATES FOR SPECIFIC PLASMODIA BY YEAR IN A. SUPERPICTUS AREAS: TIRANA, ELBASAN, BERAT, AND KAVAJA, 1932–1938

TABLE 2

Relative Frequency of Specific Plasmodial Infections by Year in Superpictus Areas: Tirana, Elbasan, Berat and Kavaja, 1932–1938

PLASMODIUM	1932	1933	1934	1935	1936	1937	1938	Total
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Falciparum	60.8	47.7	30.1	30.9	42.1	46.4	39.7	43.3
Vivax	20.6	49.4	55.5	52.9	31.6	29.7	37.0	44.0
Malariae	18.6	2.9	14.4	16.2	26.3	23.9	23.3	12.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
No. examined	1,374	3,379	3,482	2,000	2,000	2,103	2,000	

Analysis by variance of the average spleens of children exhibiting falciparum as compared with vivax parasites indicated that the differences were not significant.

DISCUSSION

Although malaria was not eliminated in either Tirana or Durazzo, it was reduced to a negligible quantity. In Tirana no infections were found among infants in either 1936 or 1937, which is evidence that transmission of the disease had either ceased

or been greatly reduced. In Durazzo poor maintenance was responsible for a recurrence of the disease in 1936 and 1937, but during the period of observation conditions in this area improved to such an extent that a new bathing beach was opened and the summer population greatly increased. As many of these people came from malarious areas they furnished a reservoir of infection for the few sacharovi entering the protected zone.

An interesting feature of the projects in both Tirana and Durazzo was that they became sources of revenue to the government, which in time would have made them self-liquidating. In Tirana the water diverted to the irrigation system was sold to the farmers desiring it. In Durazzo the inflow of fresh sea water and young fish into the lagoon produced optimum conditions for fish culture in the deeper central portion, and for a time a private company paid for the privilege of putting traps into the canal.

In areas like Valona, however, where permanent measures alone will suffice and where the cost of the undertaking is greater than the community can carry, progress must inevitably be slow. It is gratifying, therefore, to note that the amount of malaria was significantly reduced there also.

The presence of a comparison area, Kavaja, during the entire period of observation enables one to speak with assurance regarding the success of the projects in Albania. The initial level of malaria in this town was somewhat higher than that in Tirana but was below that found in Elbasan and Berat at a later date. The only biometers that decreased significantly in Kavaja were those pertaining to spleen size, and the annual rates of decrease were definitely less than those of the corresponding biometers in the protected areas.

SUMMARY AND CONCLUSIONS

Malaria surveys and antilarval measures were inaugurated in Albania in 1929 by the newly established Malaria Bureau of the Health Department and were continued under the auspices of the International Health Division of The Rockefeller Foundation through 1938. The reduction in the malaria endemic achieved in five protected towns has been appraised by statistical methods.

A. sacharovi was the malaria vector in the two coastal towns of Durazzo and Valona, while *A. superpictus* possibly assisted by maculipennis was the vector in Tirana, Elbasan, and Berat, so that antilarval measures had to be adapted to the local situation. Paris green was applied to stream beds breeding superpictus until the water could be diverted into an improved irrigation system. Sacharovi was controlled by admitting sea water to increase the salinity of the lagoons and by filling and draining the salt marshes. In Tirana and Durazzo permanent control of breeding was eventually achieved, in Elbasan and Valona a beginning was made, while in Berat temporary measures alone were employed.

Surveys of school children from 5 to 12 years of age made in February or March of each year furnish the data for the following biometers: enlarged spleen and parasite rates, the average spleen of all children and of those with and without parasites at the time of the examination as well as the average enlarged spleen, each by town and

year. Captures of anophelines at the periphery of the protected zone on an annual basis were compared with the parasite rates.

Concurrent surveys in Kavaja where no antilarval work was done furnish data which may be compared with those of the protected areas.

The results of the analysis may be summarized as follows:

1. A reduction occurred in the amount of malaria in each of the protected areas. The annual rates of decrease of the biometers were greatest in the two towns where permanent control of breeding was achieved, Tirana and Durazzo. Malaria biometers in Elbasan and Berat also decreased significantly as did the spleen indexes for Valona. In Kavaja, the comparison area, the measures of average spleen dropped less rapidly but still significantly.
2. The height of the endemic varied from area to area. It was initially higher in Elbasan, Berat, and Valona than in either Tirana, Durazzo, or the comparison area.
3. This greater malariousness was accompanied by a higher level of anopheline density throughout the period of observation.
4. In Tirana density of superpictus dropped sharply during the years 1932–1938 while that of maculipennis remained at a constant level. In Elbasan and Berat, on the other hand, density of both species and of total captures dropped similarly. In Valona no decrease either in sacharovi captures or in parasite rates was observed, and the year to year fluctuations in these two biometers were similar.
5. Fluctuations in the biometers based on surveys in Kavaja resembled those of the indexes from the superpictus areas, suggesting that a similar vector problem existed there. The less rapid decline of the biometers in this comparison area is conspicuous, however, and furnishes satisfactory evidence that the reduction in malaria in the protected towns was effected by the antilarval measures.

RESÚMEN Y CONCLUSIONES

Las encuestas maláricas y las medidas antilarvarias fueron inauguradas en Albania en 1.929 por el entonces nuevamente establecido Negociado de Malaria del Departamento de Higiene y fueron continuadas bajo los auspicios de la División Internacional de Higiene de la Fundación Rockefeller hasta 1.938. La reducción en la Malaria endémica llevada a cabo en cinco poblaciones fué avaluada por métodos estadísticos.

A. sacharovi. Fué el vector de Malaria en las dos ciudades costaneras de Durazzo y Valona, mientras que el *A. superpictus*, posiblemente ayudado por *A. maculipennis* fué el vector en Tirana, Elbasan y Berat, de tal manera que las medidas antilarvarias tuvieron que ser adaptadas a las condiciones locales. Se aplicó verde de Parías a los lechos de los arroyos, criaderos de *superpictus* hasta que el agua pudo ser manejada para un sistema de irrigación. *A. sacharovi* fué controlado subiendo la salinidad de las lagunas con agua del may y también por rellenos y por drenajes de los pantanos. En Tirana y Durazzo el control permanente de los criaderos fué, eventualmente, llevado acabo; en Elbasan y Valona éstos trabajós se comenzarén, mientras que en Berat se emplearon únicamente medidas temporales.

Las encuestas de niños escolares de 5 a 12 años de edad, hechas en Febreo o Marzo de cada año, dieron los datos para las siguientes biometrías:

Esplenomegalia y ratas parasitarias, bazo medio de todos los niños y de aquellos con y sin parásitos en el tiempo de los exámenes y también esplenomegalia media para cada ciudad y año. Capturas de Anophelinos en la periferia de la zonaprotegida sobre base anual se compararon con las ratas parasitarias.

Al mismo tiempo se hicieron encuestas en Kavaja donde no se hizo ningún trabajo antilarvario, estos datos se comparan con los datos de las áreas protegidas.

Los resultados del análisis pueden ser resumidos como sigue:

1°—Se aprecia una reducción en la cantidad de Malaria en cada una de las áreas protegidas. Las ratas anuales de decrecimiento de los biómetros fueron las mayores en las dos ciudades en las cuales se llevó a cabo trabajo de control permanente, Tirana y Durazzo. La biometría de Malaria en Elbasan y Berat también disminuyó significativamente como lo hicieron los índices esplénicos para Valona. En Kavaja, el área de comparación, las medidas de bazo medio bajaron menos rápidamente, pero también bajaron significativamente.

2°—La altura de la endemia varió de un área a la otra. Fué inicialmente más alta en Elbasan, Berat y Valona que en Tirana y Durazzo, o en el área de comparación.

3°—El más serio problema malárico fué acompañado por un nivel más alto de densidad de Anophelinos, a través del período de observación.

4°—En Tirana la densidad de *superpictus* cayó rápidamente durante los años de 1932 a 1938, mientras que la de *maculipennis*, permaneció en un nivel más o menos constante. En Elbasan y Berat, por otra parte, la densidad de ambas especies y el total de las capturas cayó de una manera similar. En Valona no se observó decrecimiento ni en las capturas de *sacharovi*, ni en las ratas parasitarias y las fluctuaciones año por año en estos dos beometros fueron también similares.

5°—Las fluctuaciones en la beometría malárica basadas en encuestas en Kavaja se parecen a aquellas de las áreas de *superpictus*, lo que sugiere que un problema similar de vector, existió allí. La menos rápida declinación de la beometría en esta área de comparación es conspicua, sinembargo, y dá evidencia satisfactoria que la reducción en la Malaria en las ciudades protegidas, fué efectuada por las medidas antilarvarias.

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EXPERIMENTAL FIELD-TYPE SUPPRESSION WITH SN 7618 (CHLOROQUINE), SN 8137 AND SN 12,837 (PALUDRINE)*

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One of the objectives of the war-time malaria research program has been to find drugs which would prove superior to quinine and quinacrine in the field-type suppression of malaria. The term "field-type suppression" is employed in this paper with reference to the periodic administration of a drug in anticipation of infection, for the purpose of suppressing the infection below the threshhold of clinical symptoms, and, if possible, parasitemia. This terminology is believed to be desirable in view of the extension in recent years of the term "suppression" to imply any effect by drugs upon erythrocytic forms, including those responsible for the acute clinical attack.

This paper will concern itself with a report of observations with experimental field-type suppression, in which a known sporozoite inoculum via mosquitoes of a standardized strain of either *vivax* or *falciparum* malaria was administered at intervals to patients under the influence of designated drugs, and an attempt was made to compare the efficacy of these drugs. These studies were carried out at the suggestion of the Panel on Clinical Testing, which also provided the drugs employed in these tests. Field trials with the drugs employed in our studies have been, and are still being, carried on in various parts of the world. Upon such trials obviously depends the final decision as to the practical value of any drug employed. However, an interpretation of the results of field trials is frequently handicapped by the variable immunity status of the population under study, as well as uncertainty with regard to the degree of mosquito infection occurring during the period of the study. For these reasons, experimental field type studies of the type herein described, in which these variables are kept at a relatively constant level, would appear to be of value in estimating the relative efficacy of drugs. Obviously, conclusions expressed in quantitative terms can apply only to the strains and species of malaria employed, as it is well-known that there are strain, as well as species, differences in the amounts of a drug required for suppression of parasitemia.

MATERIAL AND METHODS

Patient material for these studies consisted of neurosyphilitics hospitalized for malariatherapy.[1] Only white patients without a history of previous malaria were

* The work described in this paper was initiated under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Tennessee. It was completed under a grant from the United States Public Health Service

[1] The facilities of the Gailor Psychiatric Hospital, Memphis, were kindly made available to us for these studies. Dr. Y. T. Wong assisted in the clinical management of patients.

employed in these studies, in order to obtain a more critical evaluation of the effect of drugs in suppression. The McCoy strain of *P. vivax* and the Costa strain of *P. falciparum* were employed throughout our studies.

Our insectary[2] provided *Anopheles quadrimaculatus* for mosquito-induced infections, and infected mosquitoes were usually fed on test patients within 7 days after the appearance of sporozoites in the salivary glands. An attempt to estimate quantitatively the degree of infection induced was made by designating sporozoite density in each mosquito as 1, 2, 3, or 4 plus, following dissection. The aggregate of pluses for the mosquitoes fed was recorded as the total sporozoite inoculum. At least 10 pluses were considered desirable for each inoculation, as control patients have never failed to come down with malaria following such an inoculation. In many instances inocula approaching 20 pluses were given. Control patients were usually inoculated at the same time. Unless otherwise stated, patients received mosquito inoculations on three alternate days during the first week, when no priming dose of drug was given, or during the second week when an initial priming dose of drug was given one week prior to the first mosquito inoculation. In the former instance the initial dose of drug was given as a single dose the day before the first mosquito inoculation. Subsequently, the weekly amount of drug was given as a single dose at weekly intervals, on the same week day as the initial dose of drug, so that doses were given during three consecutive weeks following the week of inoculations. Drug administration was then discontinued, and patients were followed for "breakthrough". When effective suppression at a certain dosage level was achieved, the amount of drug was reduced in subsequent experiments in an attempt to determine the minimum effective weekly dosage required for suppression of parasitemia.

Thick film examinations of blood were made daily during the period of observation. Rectal temperatures were charted every four hours during the period of hospitalization. Patients were observed and questioned for evidence of toxic manifestations arising from the drug employed.

Drug dosage will be expressed in terms of base throughout this paper. The chemical structures of the drugs employed are as follows:

SN 8137	7-chloro-4-(3-diethylamino-2-hydroxypropylamino)-quinoline diphosphate
SN 7618	7-chloro-(4-diethylamino-1-methylbutylamino)-quinoline diphosphate
SN 12,837	N_1-p-chlorophenyl-N_5-isopropylbiguanide acetate

While plasma drug concentrations were determined, they will not be included in this paper, since information based upon more extensive observations than ours in this regard is available (Malaria Reports, 1945)

OBSERVATIONS IN P. VIVAX INFECTIONS

SN 8137

Twelve patients received weekly dosages ranging from 0.125 grams to 0.5 grams without a priming dose. Three control patients were inoculated with this series, and manifested customary prepatent and incubation periods. All except two

[2] The operation of this insectary was financed by a grant from the Tennessee Valley Authority.

patients (COM and THO) received three mosquito inoculations during the week following the first dose of drug. The results are summarized in Table 1. It will be observed that the minimum effective weekly suppressive dosage for SN 8137, when no priming dose is employed, lies between 0.125 and 0.25 grams. Four patients receiving the latter dosage were consistently protected, whereas four patients receiving the former dosage broke through with parasitemia and fever, although of low degree when drug administration was continued. In no case was parasitemia observed for over 5 days, and temperature elevation did not exceed 102° F. (rectal), and lasted for only 1 or 2 days at most. Toxic manifestations were not observed.

TABLE 1

Summary of Field-Type Trials with SN 8137 against P. Vivax (McCoy)

PATIENT	WEIGHT	WEEKLY DRUG DOSE IN GMS.	SPOROZOITE INOCULA			PREPATENT PERIOD DAYS	REMARKS
			1	2	3		
	kgs.						
COM	60.4	0.5	16+			—	
THO	44.7	0.5	16+			—	
HIG	70.2	Control			20+	10	
WIL	73.6	0.5	12+	16+	20+	—	
BRI	72.0	0.5	15+	16+	16+	—	
FER	80.9	0.25	16+	16+	14+	—	
CAM	64.5	0.25	19+	16+	12+	—	
BAI	69.1	Control	17+			13	
SCO	60.0	0.25	16+	14+	16+	—	
HUS	63.0	0.25	14+	16+	16+	—	
LEN	68.0	0.125	15+	16+	16+	9	(A)
BOY	51.0	0.125	14+	16+	16+	12	(B)
RAM	71.4	Control	16+			11	
EDM	70.4	0.125	16+	14+	14+	13	(C)
SCO	68.6	0.125	16+	13+	17+	25	(D)

(A) Positive thick films 9th to 14th days.
(B) Positive thick films 12th to 14th days.
(C) Positive thick film 13th day only. Fever above 102° F. on 12th and 13th days.
(D) Positive thick film one day only. Fever 102° F. on 14th day.

The four patients receiving four weekly doses of 0.5 grams broke through with clinical malaria from 120 to 270 days after the date of their first mosquito inoculation. Follow-up data upon patients receiving lower dosage were not complete enough to determine the interval elapsing before breakthrough occurred.

SN 7618 (chloroquine)

Of the six patients receiving this drug, four received priming doses, equivalent to double the weekly maintenance dose, one week prior to the week of inoculations. The remaining two did not receive a priming dose. The result are shown in Table 2. When a priming dose as described above was given, the minimal effective weekly suppressive dose lay between 0.125 grams and 0.25 grams. Two patients receiving

the former dosage broke through with parasitemia. Of interest is the fact that two patients receiving the higher dosage (0.25 grams) without a priming dose broke through with parasitemia. The importance of the priming dose is thereby emphasized, and probably reflects the fact that plasma concentrations do not become stabilized until about the third week after weekly administration of this drug is begun (Malaria Reports, 1945). None of the patients developing parasitemia manifested fever reaching a level of 102° F. (rectal). This, together with the fact that positive thick films were observed only on a single occasion indicates that

TABLE 2

Summary of Field-Type Trials with SN 7618 against P. Vivax (McCoy)

PATIENT	WEIGHT	WEEKLY DRUG DOSE IN GMS.	SPOROZOITE INOCULA			PREPATENT PERIOD DAYS	REMARKS
			1	2	3		
	kgs.						
BRE	72	0.25*	13+	16+	20+	—	
ALL	64	0.25*	17+	15+	16+	—	
CAR	50	0.125*	12+	14+	12+	—	
COU	48	0.125*	16+	16+	12+	11	Single positive thick film
MUN	40	0.25	12+	13+	12+	11	Single positive thick film
MAR	65	0.25	16+	16+	12+	9	Single positive thick film

* Preceded by a priming dose, equal to twice the weekly maintenance dose administered a week before the week of inoculation.

TABLE 3

Summary of Field-Type Trials with SN 12,837 Against P. Vivax (McCoy)

PATIENT	WEIGHT	WEEKLY DRUG DOSE IN GRAMS	SPOROZOITE INOCULA			PREPATENT PERIOD DAYS	DAYS NEGATIVE SINCE FIRST INOCULATION
			1	2	3		
	kgs.						
CAV	57	0.25	20+	16+	20+	—	73
GEO	73	0.25	20+	16+	16+	—	234
RID	54	0.087	17+	18+	18+	—	127
LAR	64	0.087	18+	16+	15+	—	145

the dosage employed was close to the minimum suppressive dose required for this particular strain. Toxic manifestations were not observed.

SN 12,837 (paludrine)

Two patients received 0.25 grams of this drug weekly, without a priming dose. No parasitemia or fever was observed during the usual period of observation.

Two patients received 0.087 grams of paludrine weekly without a priming dose. This represents one tablet of the drug, and is equivalent to 0.1 grams of the salt. Neither patient manifested parasitemia or fever during the subsequent period of observation. These results are summarized in Table 3. The minimum effective suppressive dose of paludrine against *P. vivax* (McCoy) was not determined, but it is already evident from these experiments that the amount of this drug required is no more than one-third as much as in the case of the 4-aminoquinolines studied.

OBSERVATIONS IN P. FALCIPARUM INFECTIONS

SN 7618 (chloroquine)

For comparison with the results obtained with *vivax* infections, similar experiments were carried out with *falciparum* infections, employing a dosage level which had proved adequate in suppressing *vivax* infections.

Three patients received a priming dose of 0.5 grams followed by weekly dosage of 0.25 grams. Two patients showed single positive thick films on the 7th day of incubation, while the third patient showed an elevation of temperature (103° F. rectal) on the same day, although no positive films were observed. Data on these patients are presented in Table 4.

It is evident from these observations that the *falciparum* strain employed required more drug for effective suppression than was required by the *vivax* strain. The low order of clinical activity and parasitemia observed suggests that the minimum effective suppressive dosage is not considerably above that employed.

TABLE 4

Summary of Field-Type Trials with SN 7618 against P. Falciparum (Costa)

PATIENT	WEIGHT	WEEKLY DRUG DOSE IN GRAMS	SPOROZOITE INOCULA			PREPATENT PERIOD DAYS	REMARKS
			1	2	3		
	kgs.						
KIN	52	0.25*	20+	20+	20+	—	A
RAN	81	0.25*	18+	20+	18+	7	B
HEN	49	0.25*	20+	18+	20+	7	C

* Preceded by a priming dose, equal to twice the weekly maintenance dose, administered a week before the week of inoculation.

A. Single elevation of temperature to 103° F. on 7th day. No positive films.

B. Single elevation of temperature to 102° F. on 8th day. Single positive thick film.

C. Single positive thick film.

SN 12,837 (paludrine)

These experiments were set up to assess the value of SN 12,837 against *falciparum* infections, for comparison with the results obtained with SN 7618.

Two patients received 0.5 grams weekly without a preliminary priming dose, with a single heavy mosquito inoculation the day following the first dose of drug. No parasitemia or fever was observed, nor were toxic effects evident.

Two patients received 0.25 grams weekly without priming dosage, with three mosquito inoculations during the week following the first dose of drug. No parasitemia or fever was noted in these patients during the usual period of observation. Toxic manifestations were not observed.

Five patients received 0.087 grams of paludrine weekly (1 tablet) under the same conditions as above. Three patients showed no parasitemia or fever during the usual period of observation. Two patients broke through with parasitemia and fever on the 13th and 19th days respectively after the first inoculation (Table 5).

As in the case of *P. vivax* infections, paludrine appears to exert suppressive activity

comparable to that of chloroquine against *P. falciparum* infections with weekly doses one-third as high as required when the latter drug is employed.

Since under natural conditions in a hyperendemic area infection via mosquitoes occurs at various intervals following a weekly dose of drug, an attempt was made to determine the protective effect of a single dose (0.087 grams) of paludrine given to each of a series of patients on a different day of the week following a single heavy mosquito inoculation with *falciparum* malaria. The results are indicated in Table 6. It will be observed that the specified amount of paludrine, given on any day between the first and fifth days, inclusive, following mosquito inoculation afforded

TABLE 5

Summary of Field-Type Trials With SN 12,837 Against P. Falciparum (Costa)

PATIENT	WEIGHT	WEEKLY DRUG DOSE IN GRAMS	SPOROZOITE INOCULA			PREPATENT PERIOD DAYS	DAYS NEGATIVE SINCE FIRST INOCULATION
			1	2	3		
	kgs.						
CLE	54	0.5	19+			—	233
WYN	70	0.5	15+			—	157
MIL	56	0.25	16+	13+	12+	—	194
PRI	89	0.25	17+	15+	16+	—	121
BER	57	Control		17+		9	
WHI	60	Control		16+		9	
GIB	91	0.087	16+	12+	16+	13	
MAN	74	0.087	15+	16+	16+	19	
NIC	57	0.087	15+	14+	16+	—	107
LUS	76	0.087	14+	17+	14+	—	30
BRO	74	0.087	12+	16+	16+	—	30

TABLE 6

Results of Administering One Tablet (0.087 grams) of Paludrine on Specified Days Following a Single Mosquito Inoculation With Falciparum Malaria

PATIENT	WEIGHT	SPOROZOITE INOCULUM	DAYS FOLLOWING MOSQUITO INOCULATION	RESULT
	kgs.			
LEO	60	16+	0*	Patent 16th. day
LAR	80	16+	1	No parasitemia
NEA	57	28+	2	No parasitemia
SAN	66	24+	3	No parasitemia
JAC	60	18+	4	No parasitemia
GOU	66	20+	5	No parasitemia
PER	50	20+	5	No parasitemia
MCG	62	16+	6	Patent 23rd. day
NEA	57	17+	7	Patent 21st. day
Controls				
SEL	70	20+		Patent 7th. day
BAK	60	14+		Patent 7th. day

* Drug given one hour before inoculation.

protection. Patients receiving the same amount of drug on the day of inoculation or on the sixth or seventh days broke through with parasitemia on the 16th, 23rd, and 21st days, respectively. Since plasma concentrations of paludrine following a single dose of drug of this size usually reach zero levels by the second day, the action of paludrine in this experiment must have been upon the pre-erythrocytic stages which develop from sporozoites, similar to the action we have observed in the case of plasmochin (Feldman et al., 1947). This confirms Fairley's observation that paludrine exerts a true prophylactic action in *falciparum* malaria (Personal communication). Furthermore, since erythrocytic forms of *falciparum* malaria make their appearance on the sixth and seventh days following mosquito inoculation, the failure to secure protection when paludrine was given on these respective days indicates that this drug is more active against the pre-erythrocytic than against the erythrocytic stages of the parasite, in the dosage employed. That paludrine does possess considerable activity against erythrocytic forms, however, is indicated by the prolonged prepatent periods (23 days and 21 days compared to 7 days in the control patients) observed in these instances following a single dose of drug.

The break-through occurring when paludrine was given on the sixth day following mosquito inoculation indicates that weekly dosage in this amount will probably prove inadequate for true prophylaxis of *falciparum* infections (i.e. action upon pre-erythrocytic forms). This amount of drug must be taken twice a week to secure such an effect unless higher dosage once a week proves equally effective. However, since such an effect upon pre-erythrocytic forms is not produced by any other drug employed in field-type suppression to date, it places paludrine in a unique position in this respect. Should a similar prophylactic effect be observed against *vivax* malaria, it is safe to predict that paludrine will prove superior to the 4-aminoquinolines in field-type suppression upon a large scale, in view of the low effective dosage and general absence of toxic manifestations obtained with the former drug.[3]

SUMMARY AND CONCLUSIONS

1. Under the conditions of our field-type experiments, the minimum effective weekly suppressive dose of SN 8137 required against *P. vivax* (McCoy) sporozoite-induced infections lay between 0.125 and 0.25 grams, when no priming dose was employed. No break-through occurred during the period of observation when the higher dosage was employed.

2. When equivalent dosage of SN 7618 (chloroquine) was employed under similar conditions break-through occurred at the higher level of 0.25 grams weekly. The suppressive effect of SN 7618 was enhanced by a priming dose, equal to twice the weekly maintenance dose, given one week before the week of inoculations. When such a priming dose was employed, the results obtained were similar to those mentioned above for SN 8137, i.e. the minimum effective weekly suppressive dose against *P. vivax* infections lay between 0.125 and 0.25 grams.

3. Paludrine (SN 12,837) was effective against *P. vivax* infections when a weekly dose one-third as high as that required in the case of the above 4-aminoquinolines

[3] Since this paper was submitted for publication, Fairley has reported that paludrine exerts a partial prophylactic effect against *vivax* infections. See Trans. Roy. Soc. Trop. Med. and Hyg., Vol. 40, No. 2, October 1946.

was employed, without a priming dose. The minimum effective weekly dose was not determined.

4. Against *P. falciparum* (Costa) infections, paludrine exerted suppressive activity comparable to that of chloroquine with weekly doses one-third as high as that required when the latter drug was employed.

5. The weekly doses of either chloroquine or paludrine observed to be effective in suppressing *P. vivax* infections were found to be inadequate against *P. falciparum* infections, which require higher dosage for effective suppression.

6. Evidence that paludrine is effective against the pre-erythrocytic forms of *P. falciparum* suggests that paludrine will prove superior to the 4-aminoquinolines in large scale field-type suppression, since the latter drugs do not possess this property.

RESÚMEN Y CONCLUSIONES

1º—En las condiciones de nuestro experimento tipo-campo, la mínima dosis semanal supresiva de SN8137 que se requirió contra *P. vivax* (McCoy) infecciones inducidas por esporozoítos osciló entre 0.125 y 0.25 gramos, sin dar dosis inicial. No se observaron casos positivos durante el período de observación, cuando se usó la dosis más alta.

2º—Cuando una dosis equivalente de SN7618 (Cloroquina) se empleó en condiciones similares se observaron casos positivos al nivel más alto de 0.25 gramos por semana. El efecto supresivo del SN7618 fué mejorado- dando una dosis inicial igual a dos veces la dosis de mantenimiento semanal, dada una semana antes de la semana de inoculaciones. Cuando tal dosis inicial fué empleada, los resultados obtenidos fueron similares a los mencionados para SN8137, ésto es la mínima dosis supresiva efectiva semanal contra *P. vivax*, en las infecciones fué de 0.125 a 0.25 gramos.

3º—Paludrina (SN12,837) fué efectiva contra *Pl. vivax* (infección), cuando se usó una dosis semanal correspondiente a una tercera parte de la requerida en el caso de las 4 aminoquinolines sin dosis inicial. La mínima dosis efectiva semanal no fué determinada.

4º—Contra las infecciones de *P. falciparum* (Costa) la Paludrina ejerció una actividad supresiva comparable, a la de la cloroquina, cuando se emplearon dosis semanales tan altas como una tercera parte de la requerida cuando la última droga fué empleada.

5º—Las dosis semanales de cloroquina o de Paludrina efectivas en suprimir las infecciones de *P. vivax* fueron inadecuadas contra infecciones de *P. falciparum* que requieren una dosis mayor para el efecto supresivo.

6º—La evidencia de que la Paludrina es efectiva contra las formas pre-eritrocyticas de *P. falciparum* sugiere que la paludrina se probará superior a las 4-aminoquinolinas en larga escala en el tipo de supresión de campo, desde que las últimas drogas no poséen ésta propiedad.

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